BioResearch

Lonza

Catalog 2016

Advancing Research with Biologically Relevant Solutions

official distributor

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SZABO SCANDIC

Lonza

Lonza – Leading the Evolution of Cell Culture

With over 30 years of cell culture experience, Lonza pioneered the establishment of primary cells with its Clonetics™ Primary Cells and Media product line.. These products have supported cell culture research for decades in many critical areas, including angiogenesis, cancer, respiratory and cardiovascular disease, diabetes, renal disorders and neurobiology. With cell culture moving to a third dimension for more physiological relevance, and studies showing performance differences in 3D culture versus 2D culture, Lonza continues to expand its offering, and advance cell culture to the next level with its new RAFT™ 3D Cell Culture System. Furthermore, advances in cell imaging technology provide better tools to inspect and visualize cells in culture. Lonza remains at the forefront of this with its latest CytoSMART™ System offering a cost-effective and easy to use device for monitoring your cells.

Integrated Research Solutions

We provide complete and functionally qualified solutions for your cell and molecular biology research applications to improve and accelerate experimental results. For example, you can successfully transfect our Clonetics™ Primary Cells with your gene of interest using Nucleofector™ Devices and Kits and confirm cell viability after transfection with the Vialight™ Bioassay. With our well-known and trusted leading brands for products, we continue to set the standard for quality in the industry. Integrated cell culture solutions span our product areas with guaranteed performance. Products include:

- **Clonetics™ Primary Cells and Media** – Over 150 authenticated primary cell types and optimized media, ready to use today.
- **Stem Cells and Media** – Primary adult stem and progenitor cells available with growth and differentiation media, ES-derived Motor Neurons and media, plus our new L7™ Pluripotent Stem Cell Culture System.
- **Nucleofector™ Technology** – Proven over the past 10 years as the optimal transfection technology for hard-to-transfect primary cells and cell lines.
- **RAFT™ 3D Cell Culture System** – Patented technology with complete kits for 3D tissue modeling and co-culture.
- **CytoSMART™ System** – Smart and easy live cell monitoring and imaging
- **MycoAlert™ Assay and MycoZap™ Antibiotics** – Complete portfolio for easy and quick mycoplasma detection, elimination and prevention.
- **BioWhittaker™ Media** – Classical media, plus an extensive array of serum-free media including protein-free, chemically defined and non-animal derived formulas.
- **SeaKem®, NuSieve™ and MetaPhor™ Agarose, and FlashGel™ System** for fast reliable separation and analysis of nucleic acids and proteins.

QC Testing Solutions

Lonza is the trusted QC testing solution provider for the pharmaceutical and medical device industries. We transform our practical knowledge and technical expertise to deliver a portfolio of endotoxin testing products, software and services that support the critical needs of regulated manufacturing environments, like our own.

Contacting Lonza

Purchase any of our products directly, using the contact information provided on page 11 and 12 of this catalog, or use our e-commerce capabilities at www.lonza.com/research. For technical information, please contact our Scientific Support Team. Contact information provided on page 13 of this catalog. Based on over 40 years of experience and innovation, we are committed to providing the highest quality products, services and scientific support to advance life science research with biologically relevant solutions. For all of your research and QC testing product and service needs, turn to us. We appreciate your continued support and look forward to serving you. Your Lonza BioResearch Team

Your Lonza BioResearch Team

About Lonza

Lonza is a global company serving the life-science industry. Over a century ago, we began as a small Swiss electricity company, making a few chemicals on the banks of the river Lonza in the Valais region of the Swiss Alps. More than 110 years later, we are a leading supplier to the pharmaceutical, healthcare, and life-science industries.

We offer over 4,000 products and services to more than 60,000 customers worldwide. Our customers range from professionals within the pharmaceutical, biotechnology, academic, and government research industries to manufacturers of consumer and health products, distributors, formulators, and service companies. Since 1897, we have used our enterprising character to adapt our offerings and services to your needs and to the changing technologies.

In Pharma&Biotech we partner with pharmaceutical and biopharmaceutical customers for their manufacturing needs. Using a variety of technologies, we make the ingredients used in many critical drugs, treating patients in areas such as cardiovascular diseases, cancer, neurological and infectious diseases. Cell therapy companies rely on our custom manufacturing services. And our portfolio of endotoxin, mycoplasma and microbial detection products, software and services supports the critical needs of regulated manufacturing environments.

For BioResearch we make the tools you use to develop and test therapeutics, from basic research through to final product release. These tools range from cell culture and molecular biology products for life-science research to media used in the production of therapeutics and tests for microbial detection. We offer over 2,000 research products and services, including human and animal primary cells, cell culture media and sera, transfection technologies, bioassays, as well as electrophoresis instruments and reagents.

Other markets we serve are Nutrition, Agriculture, Personal Care, Microbial Control and Materials Science.

We have a global network of sales and support offices, with representatives who are close to you, speak your language and understand your needs. Our production and R&D facilities around the world ensure the close connection necessary to best serve your local needs.

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How to Use this Catalog

This section explains some tools and hints we have included in this catalog to help you find the information you need at a glance.

Icons

The icons below refer to additional product-related information, how to reach Customer Service or Scientific Support and additional purchase details.

Great additions to our expanding research products portfolio

Additional product or technical information available online

Contact our Customer Service or Scientific Support Representatives for additional information or ordering assistance

Ordering information and instructions

Product related scientific references

Additional technical information available in the chapter “Technical Information”. Please go to the catalog page number indicated

Storage conditions

Technical Information Section

In the back of this catalog, starting on page 393, you can find the Technical Information and Sourcebook. This chapter holds additional technical information and FAQs on several of our products included in this catalog. Please watch out for this icon guiding you to the relevant page:

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

How to Order

There are several ways to order your products of choice with Lonza: via our online shop, e-mail, phone, fax or mail.

Online Ordering

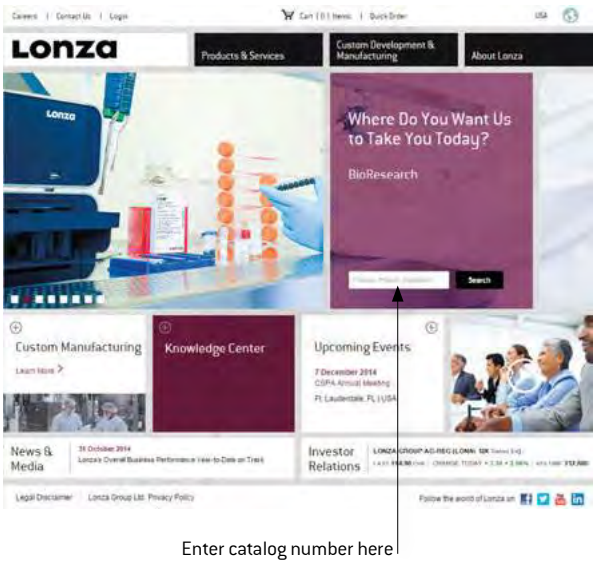
Our website encompasses an extensive product portfolio. Along with the essential tools needed to manage your electronic purchases, you will find the process simple, secure and reliable.

Our website offers:

- Highest security standards
- Ordering available 24 hours, 7 days a week
- Latest technical, application, and safety information for our products
- Sales tax, shipping charges, and expected shipping dates displayed before order is placed
- Certificates of Analysis

To order, you need a valid credit card (MasterCard®, VISA® or American Express®) or purchase order, and a shipping address. Our system will always inform you of inventory availability as you add items to your shopping basket on the top center of the website.

Go to: **http://www.lonza.com** to access our online shop. To find your product of interest, please enter the Catalog Number into the search field in the center of the webpage.



Quick Order Form

It's easy and saves time.

If you already know the Catalog Numbers and quantities of the products you would like to purchase in our online shop, you may use the Quick Order Form (see screenshot) available at **http://www.lonza.com/quickorde**r

Ordering via Ariba® or Other Third Party Providers

We support Ariba® and other third party purchasing systems through secure cXML communications. You have the choice of purchasing via a pricelist (CIF electronic catalog) or using a punch-out catalog system. We also accept and support EDI standards 850 (purchase order) and 810 (sales invoice), if your company conducts electronic transactions using EDI protocols.

Please contact us if you would like to purchase products using any of these types of electronic transmissions.

Direct Ordering via cXML

If you have developed your own internal purchasing system, you can link directly to our order entry systems via secure cXML communications protocols. Please contact us if you would like to discuss using this type of system to place your orders.

For prompt response to your e-commerce questions, please e-mail us at **ecommerce@lonza.com**.

How to Order

Continued

Ordering via E-mail, Phone, Fax or Mail

Please include all of the following information on all of your orders:

- Purchase order number
- Shipping address and billing address
- Contact name and telephone number
- Name and department of end user
- Quote or reserve lot information
- Catalog number, size, quantity, and name of products ordered

To place an order with our Customer Service Department, please use any of the following convenient options:

North America

Hours: Monday through Friday, 8:00am to 5:00pm EST

E-mail: order.us@lonza.com
Phone: +1 800 638 8174
Fax: +1 301 845 8338

Mail

Lonza Walkersville, Inc.
Customer Service Department
8830 Biggs Ford Road
Walkersville, MD 21793 USA

Europe

Hours: Monday through Friday , 8:30am to 5:30pm CET

E-mail: order.europe@lonza.com

Customer Service European Office Numbers

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Luxemburg	+32 87 321 611	+32 87 321 634
The Netherlands	0800 022 4525	0800 022 4942
United Kingdom	0808 2349 788	0808 2349 778

Mail

Lonza Verviers, S.p.r.l.
Parc Industriel de Petit-Rechain
4800 Verviers, Belgium

Ordering Information – Outside the U.S. and Europe

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India	+91 22 4342 4000	+91 22 4342 4050
	E-mail: scientificsupport.india@lonza.com	
Japan	+81 3 5566 0612	+81 3 5566 0619
	E-mail: scientific.support.jp@lonza.com	
Singapore	+65 6521 4379	+65 6521 4378
	E-mail: bioscience.asia@lonza.com	

If your country is not listed above, please check the current list of Lonza global distributors on our website:
www.lonza.com/dist

In the event that you do not find an authorized distributor for your country in the list, please contact:

Lonza Walkersville, Inc.

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- www.lonza.com/faq
  Frequently asked questions are answered online in our knowledgebase

- www.lonza.com/celldatabase
  Find your cell of interest in our Cell Database

- www.lonza.com/citations
  Thousands of scientific references can be viewed in our Citation Database

- www.lonza.com/technical-library
  This Knowledge Center holds the latest technical information, troubleshooting tips, and protocols on products and applications as well as full product information, instructions, Certificates of Analysis and Safety Data Sheets available

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Phone: +1 800 521 0390
E-mail: scientific.support@lonza.com

Phone from outside the US: +1 301 898 7025

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U.S. Patents

5,641,626**	6,512,236	6,365,341	6,117,293
6,464,850	6,198,107	6,558,521	D510,770
6,599,711	6,328,870	6,914,250	7,320,859
D511,386	D524,449	5,486,359**	
7,332,332	5,385,839	6,905,585**	

European Patents:

1297119	1383901	1390518	1476537
1525900	1522587	1702677	1741778
PCT/EP01/07348	PCT/DE02/01489	PCT/DE02/01483	

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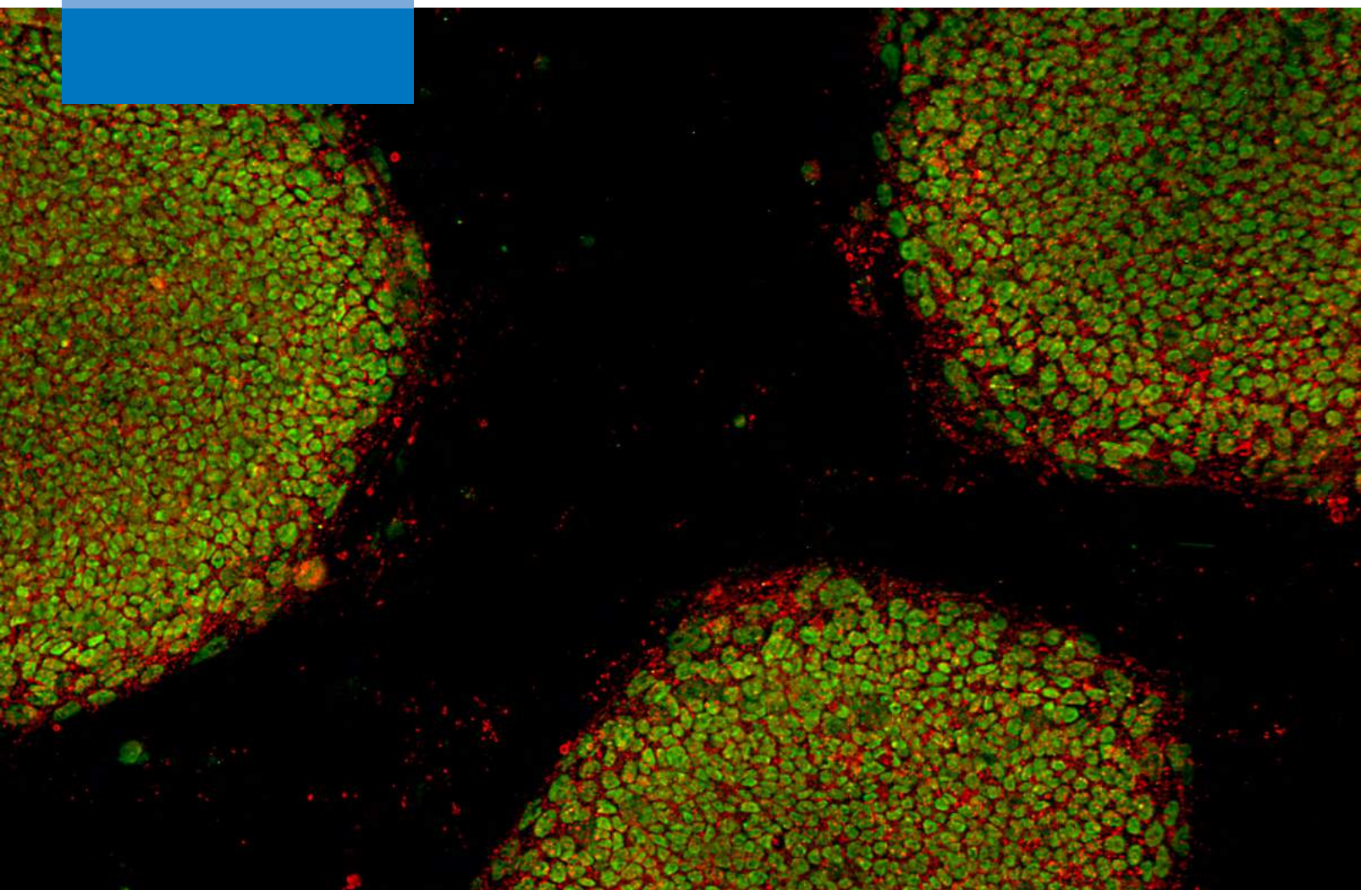
1 Stem Cells and Media



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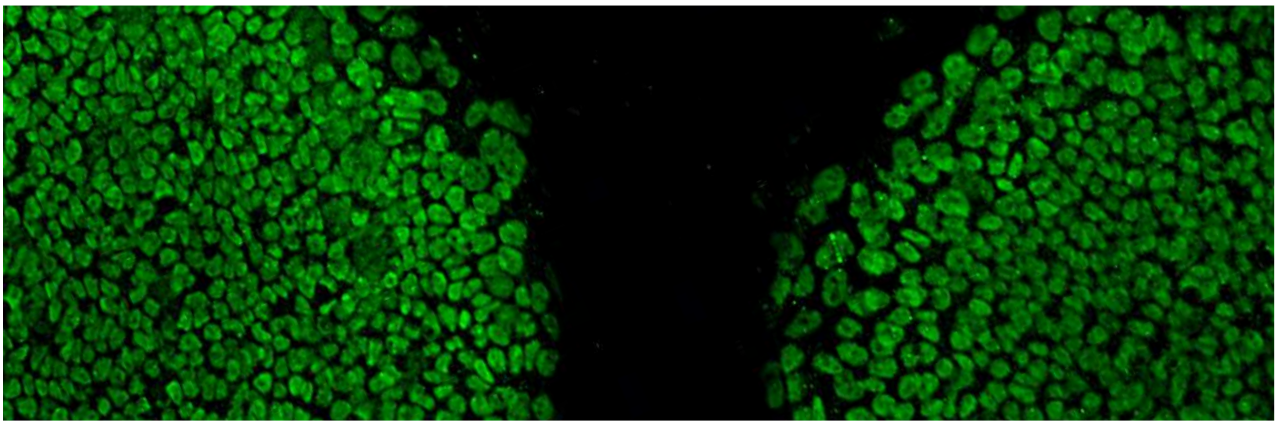
Stem Cells and Media

We do the isolation, you do the research



Stem Cells and Media

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Introduction

By definition, human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are pluripotent cells that have the ability to indefinitely self-renew and become any cell type in the body. Because of these attributes, PSCs have become an important scientific tool and are spurring advancements in basic research, disease modeling, drug development, and regenerative medicine. No longer do researchers have to rely on ethically plagued embryonic stem cells. With the revelation of induced pluripotency in 2007, there is a new avenue for therapeutic use of stem cells via allogenic and autologous methods.

Ten years ago, stem cell researchers were studying how bone marrow transplants could be used to cure blood diseases, such as leukemia and sickle cell anemia. Now with the advances in stem cell research and induced pluripotency, more patient-specific therapies are being explored. Autologous hiPSC–derived retinal pigment epithelium cell sheets can be used to treat patients with age-related macular degeneration. Allogenic treatment of MSC's have shown great promise in helping patients afflicted with irritable bowel syndrome. Autologous use of adult stem cells differentiated down the chondrogenic lineage can be used to aid in patients with osteoarthritis. While clinical use of stem cells has shared its part in many ethical debates, promising work is being done to meet patients' needs for generalized and specialized treatments.

To aid in multiple facets of stem cell research, Lonza offers an expansive portfolio of products that will help simplify your reprogramming, proliferation and differentiation of stem cells. From Lonza's new L7™ hiPSC Reprogramming and hPSC Culture system, to highly published adult stem cells and Nucleofector™ Kits for genome editing and reprogramming; Lonza offers a variety of products to support your research and improve your workflow.

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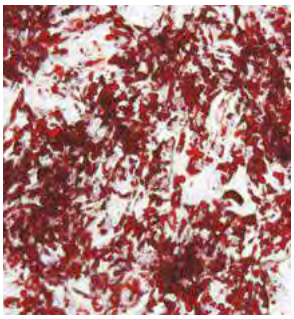
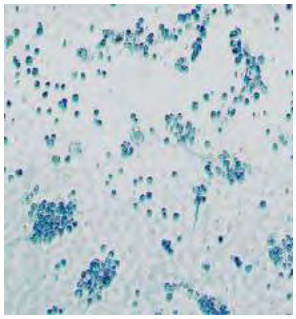
Human Adipose-derived Stem Cells (ADSC) and Media

Our human ADSCs are isolated from adipose tissue from normal, or Type I, or Type II diabetic donors. Cells can be selected from lots based on donor characteristics such as age, sex, race, and BMI.
The cells are cryopreserved at primary passage and have been reported in multiple publications to differentiate down various lineages including chondrogenic, osteogenic, adipogenic, myogenic, neural, and endothelial. ADSC Growth Medium BulletKit™ has been optimized for cell maintenance and expansion.



ADSC – derived adipocytes stained with Oil Red O



ADSC – derived chondrocytes stained with Alcian Blue

ADSC – derived osteoblasts stained with Alizarin Red

Applications

- Toxicology/drug screening
- Regenerative medicine/cell therapy
- Obesity
- Osteoporosis
- Cardiovascular disease
- Metabolic disorders

Specifications

- ≥1 million viable cells after thaw; >95% pure
- Positive for CD13, CD29, CD44, CD73, CD90, CD105, CD166, surface markers
- Negative for CD14, CD31, CD45, surface markers
- Negative for HIV-1, Hepatitis B and C
- Guaranteed to expand through five passages

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
PT-5006	PT-5006	hADSC – Human Adipose-Derived Stem Cells	Cryopreserved	≥1 million cells/vial
Diseased Cells				
PT-5007	PT-5007	D-hADSC – Diseased Human Adipose-derived Stem Cells – Diabetes Type I	Cryopreserved	≥1 million cells/vial
PT-5008	PT-5008	D-hADSC – Diseased Human Adipose-derived Stem Cells – Diabetes Type II	Cryopreserved	≥1 million cells/vial

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-4505	PT-4505	ADSC – Apidose-Derived Stem Cells Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
PT-3273	PT-3273	ADSC – Apidose-Derived Stem Cells Basal Medium		500 mL
PT-4503	PT-4503	ADSC – Apidose-Derived Stem Cells Growth Medium SingleQuots™ Supplements and Growth Factors	Frozen supplements	Kit

Related Products	Page
OsteoImage™ Mineralization Assay	275
PGM™ 2 Preadipocyte Growth Medium-2 BulletKit™	28
hMSC – Human Mesenchymal Stem Cells	29
Nucleofector™ Kits for Primary Stem Cells	226

Human Bone Marrow Products

Unprocessed Bone Marrow

Fresh, unprocessed bone marrow is collected Monday through Thursday. Orders are shipped the same day as the bone marrow is drawn with overnight delivery.

Our high quality human bone marrow is guaranteed to contain a minimum or 15 million nucleated cells per milliliter (mL).

Please contact your Scientific Support Specialist for further details on ordering fresh marrow.

Human Stromal Cells

When bone marrow cells are put into culture under specific conditions, a stromal layer of adherent cells develops over a few weeks. The stromal layer is composed of many cell types, including fibroblasts, mesenchymal stem cells, adipocytes, endothelial cells and macrophages. These stromal cells function as a feeder layer for hematopoietic progenitors, allowing proliferation and differentiation of progenitors to continue for weeks in these cultures with no addition of exogenous cytokines. This long-term culture system allows researchers to study an *in vitro* model of the bone marrow microenvironment, including cell-cell interactions, adhesion molecules and cytokine secretion. These and many other factors allow for the tight regulation of blood cell production, progenitor cell commitment and differentiation, and stem cell renewal.

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
1W-500	1W-500	Autologous Peripheral Blood*	Fresh	100 mL
1M-125	1M-125	Unprocessed Human Bone Marrow	Fresh	25 mL
Order 4 × 1M-125		Unprocessed Human Bone Marrow, same donor	Fresh	100 mL
1M-105	1M-105	Unprocessed Human Bone Marrow	Fresh	10 mL
2M-302	2M-302	Human Bone Marrow Stromal Cells	Cryopreserved, non-irradiated	≥5 million cells/vial

*Peripheral blood can only be ordered in combination with unprocessed bone marrow from the same donor

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Human Mononuclear Cells

Human Bone Marrow Mononuclear Cells

Bone marrow is the primary source of hematopoietic progenitors. These progenitors can be readily isolated from the mononuclear cell fraction. Bone marrow mononuclear cells are available from a large and varied donor pool.

Bone marrow mononuclear cells are prepared by centrifugation in a density cell separation medium (Ficoll-Paque®, GE HealthCare Bio-Sciences AB). Isolating the mononuclear cells eliminates erythrocytes and granulocytes, producing a more stable cell product. Mononuclear cells can be used directly in hematopoietic assays, or as the starting material for isolating $CD34^+$ progenitor cells. Cells are available fresh or cryopreserved. When shipped fresh, cells are suspended in HBSS containing 0.5% BSA and 5 mM EDTA.

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
1M-125A	1M-125A	Human Bone Marrow Mononuclear Cells	Fresh	≥200 million cells/vial
1M-125C	1M-125C	Human Bone Marrow Mononuclear Cells	Fresh	≥25 million cells/vial
1M-125D	1M-125D	Human Bone Marrow Mononuclear Cells	Fresh	≥100 million cells/vial
1M-125E	1M-125E	Human Bone Marrow Mononuclear Cells	Fresh	≥300 million cells/vial
2M-125C	2M-125C	Human Bone Marrow Mononuclear Cells	Cryopreserved	≥25 million cells/vial
2M-125E	2M-125E	Human Bone Marrow Mononuclear Cells	Cryopreserved	≥300 million cells/vial
2S-101D	2S-101D	Human Bone Marrow Mononuclear Cells	Cryopreserved	≥5 million cells/vial

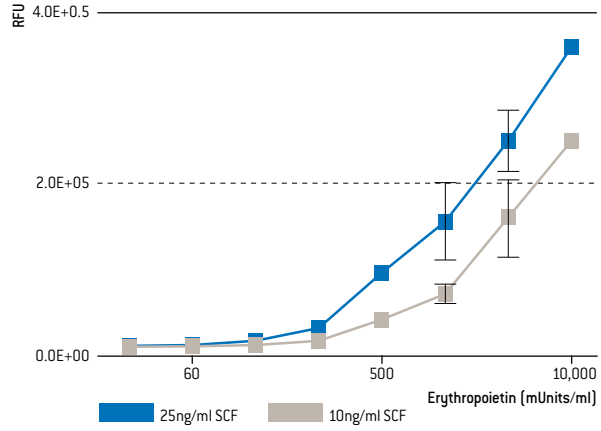
Related Products	Page
HPGM™ Hematopoietic Progenitor Growth Medium	23

Human CD34⁺ Progenitor Cells

CD34⁺ progenitor cells are available from bone marrow and cord blood. The cells are isolated from mononuclear cells using positive immunomagnetic selection and cell purity via FACS is >90%. CD34 is a known marker for hematopoietic progenitor cells. As these cells mature and differentiate, CD34 expression is lost.

Fresh cells need to be scheduled 2 weeks in advance depending on the source and volume requirements.

Erythropoiesis from CD34⁺ Progenitor Cells



Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
1M-101	1M-101	Human Bone Marrow CD34⁺ Progenitor Cells	Fresh	≥100,000 cells/vial
1M-101A	1M-101A	Human Bone Marrow CD34⁺ Progenitor Cells	Fresh	≥300,000 cells/vial
1M-101B	1M-101B	Human Bone Marrow CD34⁺ Progenitor Cells	Fresh	≥500,000 cells/vial
1M-101C	1M-101C	Human Bone Marrow CD34⁺ Progenitor Cells	Fresh	≥1 million cells/vial
2M-101	2M-101	Human Bone Marrow CD34⁺ Progenitor Cells	Cryopreserved	≥100,000 cells/vial
2M-101A	2M-101A	Human Bone Marrow CD34⁺ Progenitor Cells	Cryopreserved	≥300,000 cells/vial
2M-101B	2M-101B	Human Bone Marrow CD34⁺ Progenitor Cells	Cryopreserved	≥500,000 cells/vial
2M-101C	2M-101C	Human Bone Marrow CD34⁺ Progenitor Cells	Cryopreserved, volume discount available	≥1 million cells/vial
2M-101D	2M-101D	Human Bone Marrow CD34⁺ Progenitor Cells	Cryopreserved	≥2 million cells/vial
2M-101F	2M-101F	Human Bone Marrow CD34⁺ Progenitor Cells	Cryopreserved	≥10 million cells/vial
2C-101	2C-101	Human Cord Blood CD34⁺ Progenitor Cells	Cryopreserved	≥1 million cells/vial
2C-101A	2C-101A	Human Cord Blood CD34⁺ Progenitor Cells	Cryopreserved	≥500,000 cells/vial
2C-101B	2C-101B	Human Cord Blood CD34⁺ Progenitor Cells	Cryopreserved	≥100,000 cells/vial

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Human CD34$^+$ Medium

Hematopoietic Progenitor Growth Medium (HPGM™) is a serum-free medium containing only human proteins that supports the proliferation and differentiation of hematopoietic progenitors with the addition of cytokines.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Size
PT-3926	PT-3926	HPGM™ Hematopoietic Progenitor Growth Medium	500 mL

Related Products	Page
Human CD34$^+$ Progenitor Cells	22
Human Mononuclear Cells	21
Nucleofector™ Kits for Human CD34$^+$ Cells	224
Iscove's Modified Dulbecco's Medium (IMDM)	113

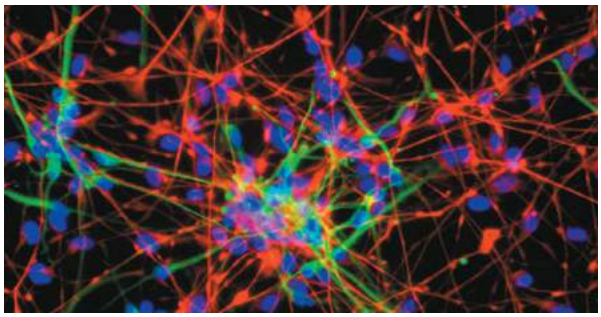
Human Neural Progenitor Cells (NHNP) and Media

Poietics™ Neural Progenitor Cells are cryopreserved as neurospheres isolated from human brain cortex. Lonza offers 2 optimized media kits specially formulated to support the maintenance and differentiation of the NHNP cells.

NPMM™ Neural Progenitor Maintenance Medium BulletKit™ contains the necessary supplements and media for optimal NHNP cell maintenance. This kit includes:
- CC-3210 – Neural Progenitor Basal Medium
- CC-4241 – Neural Progenitor Maintenance Medium SingleQuots™ Kit (contains hEGF and hFGF)
- CC-4242 – Neural Progenitor Supplement SingleQuots™ Kit (contains NSF-1 and GA)

NPDM™ Neural Progenitor Differentiation Medium BulletKit™ contains the necessary supplements and media for optimal NHNP differentiation. The medium can be customized by supplementation with differentiation-promoting agents such as brain-derived neurotrophic factor. This kit includes:
- CC-3210 – Neural Progenitor Basal Medium
- CC-4242 – Neural Progenitor Supplement SingleQuots™ Kit

Benefits
- Cryopreserved in primary passage
- Guaranteed marker expression when plated onto laminin
- Media to support maintenance and differentiation



NHNP stained for -tubulin III and GFAP

Applications
- Drug development
- Neurotoxicity
- Neurogenesis and CNS function
- Neurotransmitter disorders
- Electrophysiology
- Regenerative medicine

Cell Testing and Specifications
- Cells and media tested together for optimal performance
- Cells negative for HIV-1, Hepatitis B and C
- Cells negative for mycoplasma, bacteria, yeast and fungi
- Cells test positive for β-tublin III and GFAP

1. **Sold under license from StemCells, Inc.** US patents 5,968,829 and 5,851, 832.

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-2599	PT-2599	NHNP – Human Neural Progenitor Cells	Cryopreserved	≥1.2 million cells/vial

For cell pellets in RNALater contact Customer Service for order placement..

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3209	CC-3209	NPMM™ Neural Progenitor Maintenance Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3210	CC-3210	NPBM™ Neural Progenitor Basal Medium		200 mL
CC-3229	CC-3229	NPDM™ Neural Progenitor Differentiation Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4241	CC-4241	NPMM™ Neural Progenitor Differentiation Maintenance Medium SingleQuots™ Supplements	Frozen supplement	Kit
CC-4242	CC-4242	Neural Progenitor SingleQuots™ Supplements	Frozen Supplement	Kit

See page 400–408.

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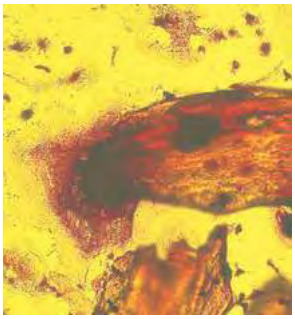
Human Osteoclast Precursor Cells (OCP) and Media

- **Poietics™ Osteoclast Precursor Cells and Media System includes:**
  - Cryopreserved human osteoclast precursors
  - OCP Osteoclast Precursor Medium BulletKit™

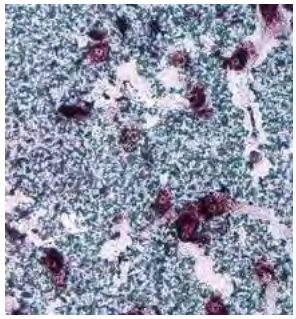
Osteoclasts are large, multinucleated cells that play an active role in bone resorption. This cell system has been designed for use in high-throughput applications to conduct research on osteoporosis, bone resorption, and other bone-related diseases.

OCP Osteoclast Precursor Medium BulletKit™ includes the basal medium and supplements needed to induce the osteoclast precursors to differentiate into mature osteoclasts; these differentiated osteoclasts stain positive for TRAP and express the calcitonin receptor.

Poietics™ Cells, Media, and Reagents are quality tested together and guaranteed to give optimum performance as a complete cell system.

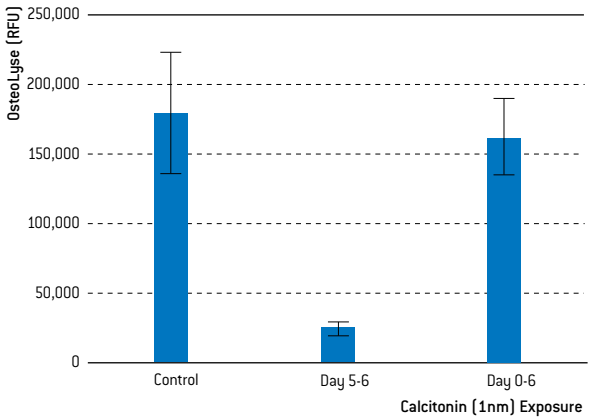


Differentiated human osteoclasts resorbing bone fragment



Pits formed from bone resorption activity of differentiated osteoclasts

Effects of Calcitonin on Osteoclast-mediated Bone Matrix Degradation *in vitro*



Inhibition of bone matrix resorption by calcitonin. Poietics™ Primary Human Osteoclast Precursors were cultured in differentiation medium containing no calcitonin, calcitonin added only at day 5 and calcitonin added on days 0 and 5 and assayed after a total of 6 days. Calcitonin, added at day 0, resulted in the osteoclasts becoming refractory to calcitonin added on day 5.

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
2T-110	2T-110	hOPC – Human Osteoclast Precursor Cells	Cryopreserved	≥1 million cells/vial

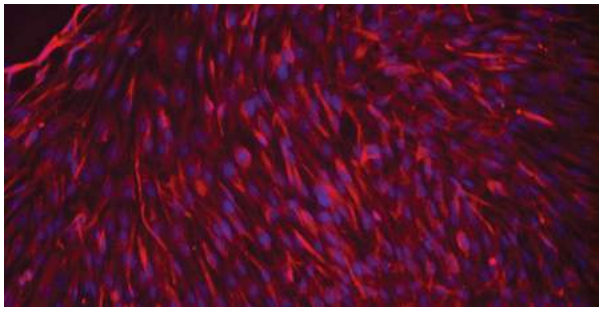
Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-8001	PT-8001	OCP – Osteoclast Precursor Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
PT-8201	PT-8201	OCP – Osteoclast Precursor Basal Medium		100 mL
PT-9501	PT-9501	OCP – Osteoclast Precursor Medium SingleQuots™ Supplements	Frozen supplements	Kit

Related Products	Page
OsteoAssay™ Human Bone Plate	273
OsteoImage™ Mineralization Assay	275
OsteoLyse™ Assay Kit	274

Dental Pulp Stem Cells (DPSC) and Media

Dental Pulp Stem Cells (DPSC) are multipotent stem cells harvested from the soft living tissue inside adult teeth. Cryopreserved for ease of use and experimental flexibility, these Mesenchymal-like stem cells have the potential to differentiate into a variety of cell types including osteoblasts, adipocytes, chondrocytes and neurons. Lonza also offers DPSC Media BulletKits™, specifically optimized for cell maintenance and expansion. Each kit contains the necessary media and supplements for maximum cell growth and rapid expansion.



Dental pulp stem cells stained for dentin sialophosphoprotein (DSPP) (red) and DAPI (blue)

Source
- Adult human third molars

Applications
- Comparative stem cell studies
- Wound healing
- Stem cell differentiation
- Tissue regeneration
- Muscular Dystrophy research

Cell Testing and Specifications
- ≥ 1 million viable cells
- Guaranteed to expand through 5 passages
- Negative for HIV-1. Hepatitis B and C
- Positive for CD166, CD105, CD90, CD73 and CD29 surface markers
- Negative for CD133, CD45 and CD34 surface markers passages

Marker Tested via Flow Cytometry	Results
CD166	>95%
CD105	>95%
CD29	>95%
CD90	>90%
CD73	>90%
CD45	<10%
CD34	<10%
CD133	<10%

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-5025	PT-5025	Human Dental Pulp Stem Cells	Cryopreserved	≥1 million cells/vial

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-3005	PT-3005	Human Dental Pulp Stem Cell BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
PT-3927	PT-3927	Human Dental Pulp Stem Cell Basal Medium		500 mL
PT-4516	PT-4516	Human Dental Pulp Stem Cell SingleQuots™ Supplements and Growth Factors	Frozen supplements	Kit

See page 400–408.

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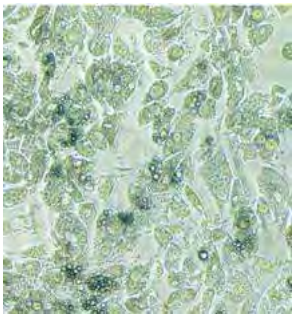
Human Preadipocyte Cells and Media

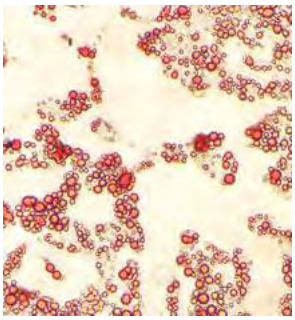
Poietics™ Preadipocyte Cells are isolated from subcutaneous or visceral fat. Subcutaneous fat is often found attached to skin in the lower abdomen area. Visceral preadipocytes are isolated from adipose tissue associated with internal organs, such as the bladder or kidney.

Relative to subcutaneous fat, visceral fat deposits are mobilized at a higher rate to produce serum fatty acids which contribute to insulin resistance, Diabetes Type 2, and other related cardiovascular disorders.

Preadipocytes are precursor cells that develop into adipocytes when fully differentiated. Adipocytes perform essential functions of energy metabolism and are characterized by the accumulation of intracellular triglycerides.



Differentiated subcutaneous preadipocyte cells

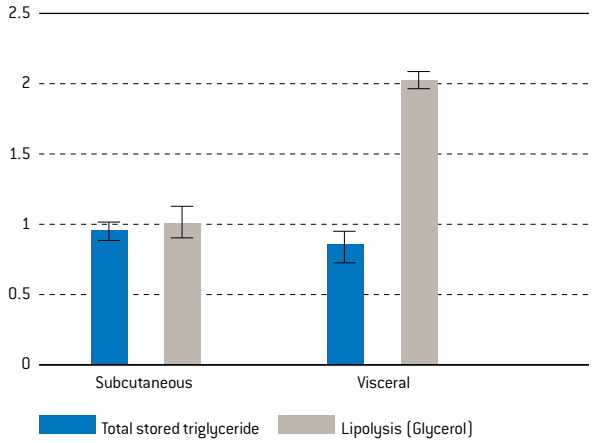


Differentiated visceral preadipocyte cells stained with Oil Red O

Poietics™ Preadipocyte Cells and Media System includes:

- Cryopreserved human preadipocyte cells isolated from subcutaneous or visceral fat
- Cells available from normal, Type I, or Type II diabetic donors
- PGM™ 2 Preadipocyte Growth Medium-2 BulletKit™, which contains the basal medium and growth supplements needed to induce growth and differentiation of the preadipocytes into mature adipocytes
- AdipoRed™ Assay Reagent, an assay reagent for high-throughput quantification of intracellular lipid

Catecholamine-induced Lipolysis in Subcutaneous and Visceral Primary Human Preadipocytes



Applications

- Lipid accumulation and metabolism
- Obesity
- Diet drug development
- Diabetes research
- Insulin sensitivity

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
PT-5001	PT-5001	Human Subcutaneous Preadipocyte Cells	Cryopreserved	≥4 million cells/vial
PT-5005	PT-5005	Human Visceral Preadipocyte Cells	Cryopreserved	≥1 million cells/vial
PT-5020	PT-5020	Human Subcutaneous Preadipocyte Cells	Cryopreserved	≥1 million cells/vial
Diseased Cells				
PT-5021	PT-5021	Diseased Human Subcutaneous Preadipocyte Cells – Diabetes Type I	Cryopreserved	≥1 million cells/vial
PT-5022	PT-5022	Diseased Human Subcutaneous Preadipocyte Cells – Diabetes Type II	Cryopreserved	≥1 million cells/vial
PT-5023	PT-5023	Diseased Human Visceral Preadipocyte Cells – Diabetes Type I	Cryopreserved	≥1 million cells/vial
PT-5024	PT-5024	Diseased Human Visceral Preadipocyte Cells – Diabetes Type II	Cryopreserved	≥1 million cells/vial

Human Preadipocyte Cells and Media

Continued

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-8002	PT-8002	PGM™ 2 Preadipocyte Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
PT-8202	PT-8202	PBM™ 2 Preadipocyte Basal Medium-2	Contains insulin	500 mL
PT-9502	PT-9502	PGM™ 2 Preadipocyte Growth Medium SingleQuots™ Supplements and Growth Factors	Frozen supplements	Kit
PT-8200	PT-8200	PBM™ Preadipocyte Basal Medium	Insulin-free	500 mL
17-512F	BE17-512F	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) without calcium or magnesium	500 mL

See page 400–408.

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Human Mesenchymal Stem Cells (hMSC) and Media

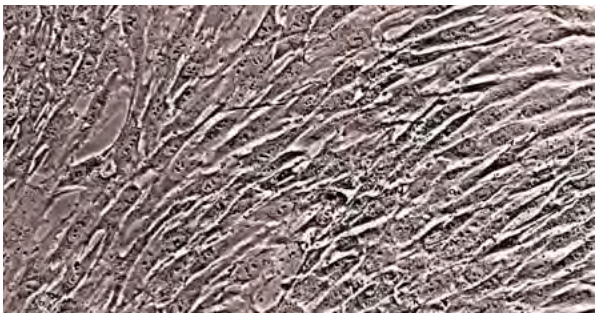
Bone marrow contains a population of rare progenitor cells known as mesenchymal stem cells (MSC) capable of replication as undifferentiated cells or differentiating into bone, cartilage, fat, muscle, tendon and marrow stroma. Poietics™ Human Mesenchymal Stem Cell System contains normal human mesenchymal stem cells and medium for their growth and differentiation. Cells are frozen after passage two and it is recommended that experiments are performed by passage five.

Each system can generate hMSC cultures for experimental studies in cell differentiation, including osteogenesis and bone mineralization, chondrogenesis and cartilage formation, adipogenesis and fat accumulation. They are excellent models for gene delivery research, functional genomics, drug screening, high-throughput screening and toxicology.

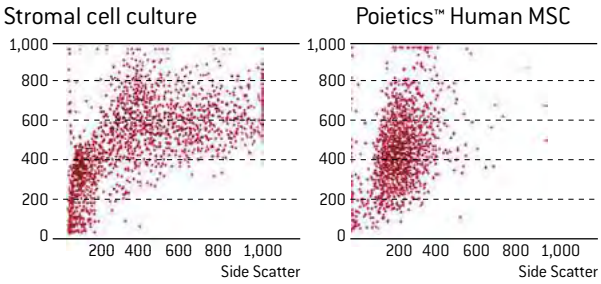
Poietics™ Cells and Media are quality tested and guaranteed to give optimum performance. hMSCs are tested using *in vitro* assays and are found to be positive for: adipogenic lineage as indicated by AdipoRed™ Assay Reagent lipid stain; chondrogenic lineage as indicated by TGF-beta staining and osteogenic lineage as indicated by OsteoImage™ Mineralization Assay stain of calcium deposition.

Cell Testing and Specifications

- HIV-1, Hepatitis B and Hepatitis C are not detected in all donors
- Cells are tested for purity by flow cytometry:
- Cells are positive for CD105, CD166, CD29, and CD44
- Cells are negative for CD14, CD34, and CD45
- Tested for the ability to differentiate into osteogenic, chondrogenic, adipogenic lineages



hMSC in culture



Mesenchymal stem cell differentiation kits are licensed by Lonza from Osiris Therapeutics, Inc. and are subject to the following limited use license:

The included biological material, including progeny and derivatives, (collectively referred to as Material) is licensed to you under specific terms. You are responsible for ensuring that the terms of the license agreement are met.

1. **Grants of License: Lonza Walkersville, Inc.** grants you a non-transferable, non-exclusive license to use the Material for research.
2. **Not for Human Use: The Material may not be used: a) in humans; b) in conjunction with human clinical trials; c) in association with human diagnostics.**
3. **Material Not Transferable: You may not transfer the Material to any other person or organization.**
4. **Patent Notice: Material under license from Osiris Therapeutics, Inc.** Material is covered by US patent 5,486,359 and others.

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-2501	PT-2501	hMSC – Human Mesenchymal Stem Cells	Cryopreserved	≥750,000 cells/vial

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-3001	PT-3001	MSCGM™ Mesenchymal Stem Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
PT-3002	PT-3002	hMSC – Human Mesenchymal Stem Cell Osteogenic Differentiation Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
PT-3003	PT-3003	hMSC – Human Mesenchymal Stem Cell Chondrogenic Differentiation Medium BulletKit™	Includes basal medium and SingleQuots™ Kit, TGF-β3 sold separately	Kit

Human Mesenchymal Stem Cells (hMSC) and Media

Continued

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
PT-3004	PT-3004	hMSC – Human Mesenchymal Stem Cell Adipogenic Differentiation Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
PT-3238	PT-3238	MSCBM™ Mesenchymal Stem Cell Basal Medium		440 mL
PT-4105	PT-4105	MSCGM™ Mesenchymal Stem Cell Growth Medium SingleQuots™ Supplements and Growth Factors	Frozen supplements	Kit
PT-4124	PT-4124	rhTGF-β3 for hMSC Chondrogenic Differentiation Medium Supplement		2 µg
190632	190632	MSCGM™ CD Mesenchymal Stem Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
190620	190620	MSCBM™ CD Mesenchymal Stem Cell Basal Medium – Chemically Defined		500 mL
192125	192125	MSCGM™ CD Mesenchymal Stem Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit

Ordering Information – Reagents

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
17-512F	BE17-512F	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) without calcium or magnesium	500 mL
CC-3232	CC-3232	Trypsin/EDTA for Mesenchymal Stem Cells		100 mL

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Adipolyze™ Lipolysis Detection Assay	272
Nucleofector™ Kits for Human Mesenchymal Stem Cells	226

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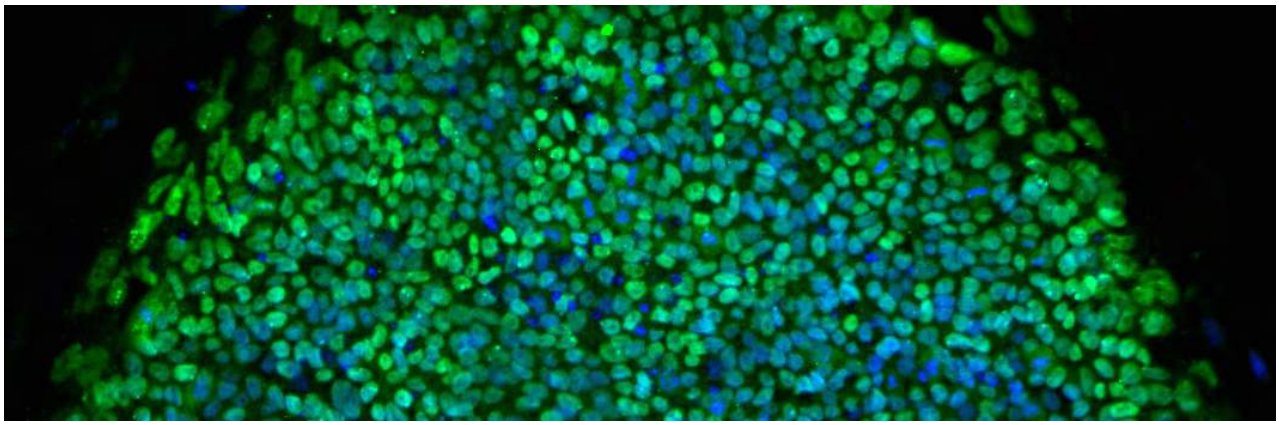

Pluripotent Stem Cells and Media

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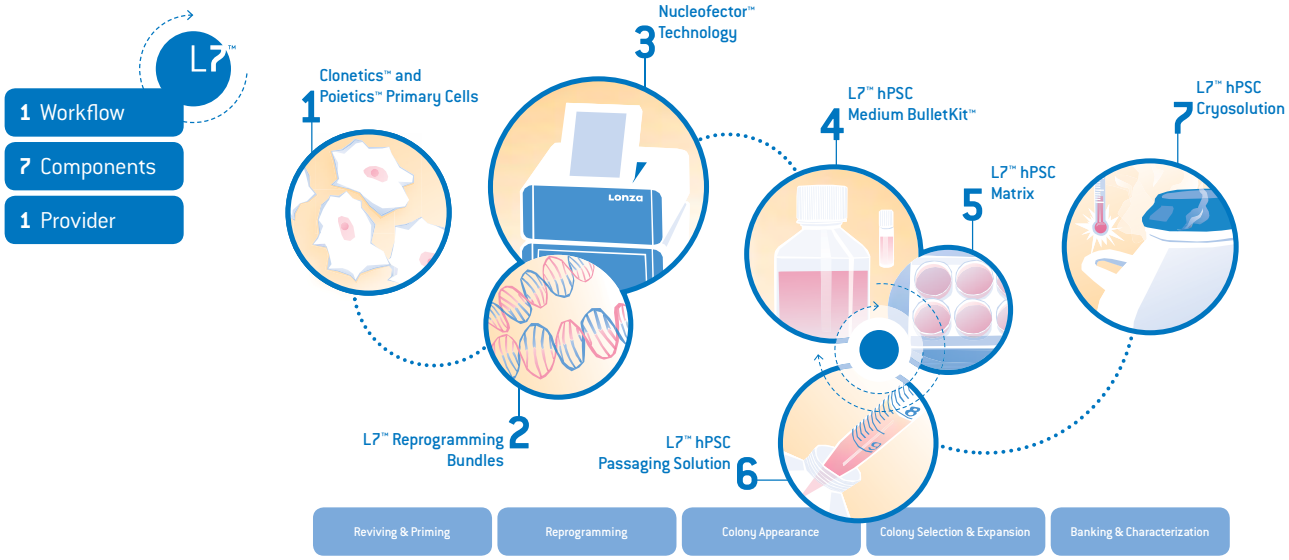
Pluripotent Stem Cells

In 1998, Dr. James Thompson's laboratory at the University of Wisconsin was the first to successfully isolate and culture human embryonic stem cells (hESCs) *in vitro*. In 2007, Dr. Shinya Yamanaka and colleagues at Kyoto University became the first to successfully convert adult human cells to an embryonic stem cell-like state or induced pluripotent stem cells (hiPSCs). Five years later, Dr. Yamanaka was awarded a Nobel Prize for this work.

By definition, hESCs and hiPSCs have the ability to indefinitely self-renew and become any cell type in the body. Because of these attributes, PSCs have become an important scientific tool and are spurring advancements in basic research, disease modeling, drug development, and regenerative medicine.

L7™ Overview

In 2011, Lonza began development on a clinical grade master cell bank for NIH. During development, it was determined that existing commercial products did not provide the optimal xeno-free, defined conditions needed for hPSC culture. As a result, the L7™ hiPSC Reprogramming and hPSC Culture System was created. The resulting system is comprised of a culture platform (medium, matrix, subculture reagent, and cryopreservation medium) that supports every-other-day feeding of human PSCs in a defined system. This culture system used in combination with an enhanced episomal reprogramming method generates hiPSCs derived from blood cells that demonstrate the hallmarks of human pluripotent stem cells.



For more information on L7™ hiPSC Reprogramming see page 36 and 228.

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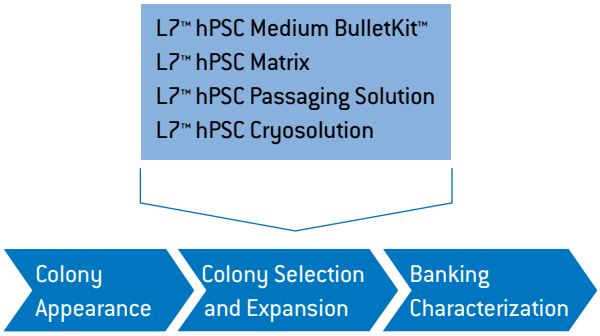
L7™ hPSC Culture System

The L7™ hPSC Culture System is a robust culture system that supports every other day feeding of pluripotent stem cells. This xeno-free, fully defined system includes a medium, matrix, passaging and cryosolution. The L7™ System is a complete solution for the maintenance and expansion of human ESCs and iPSCs



Benefits

- Robust performance and compatibility with most lines
- Reduced workload and increased flexibility by supporting every-other-day feeding
- Efficient differentiation into all three germ layers
- Easy transition to clinical applications

Ordering Information – L7™ hPSC Culture System

Cat. No. NA	Cat. No. EU	Description	Description	Size
FP-5013	FP-5013	L7™ hPSC Passaging Solution		100 mL
FP-5002	FP-5002	L7™ hPSC Cryosolution		50 mL
FP-5020	FP-5020	L7™ hPSC Matrix	Sufficient for coating up to 1,000 cm^2 of culture vessel surface area	1 mg
FP-5007	FP-5007	L7™ hPSC Media BulletKit™	Includes basal medium and SingleQuots™ Supplement	500 mL Basal medium + 5 mL Supplement

For additional information on the L7™ hPSC Culture System, visit: www.lonza.com/L7

L7™ PBMC Reprogramming Bundles

The Nucleofector™ Technology has been demonstrated to be an efficient and cost-effective non-viral alternative for iPSC generation and is being used by leading scientists around the world. It has been successfully tested for reprogramming various cell types, including PBMCs, fibroblasts, or CD34⁺ hematopoietic stem cells.

Using the 4D-Nucleofector™ System, Lonza's Pluripotent Stem Cell Innovation Team has developed an optimized protocol for reprogramming of peripheral blood mononuclear cells (PBMCs) using episomal vectors. Components required to run the optimized protocol are:

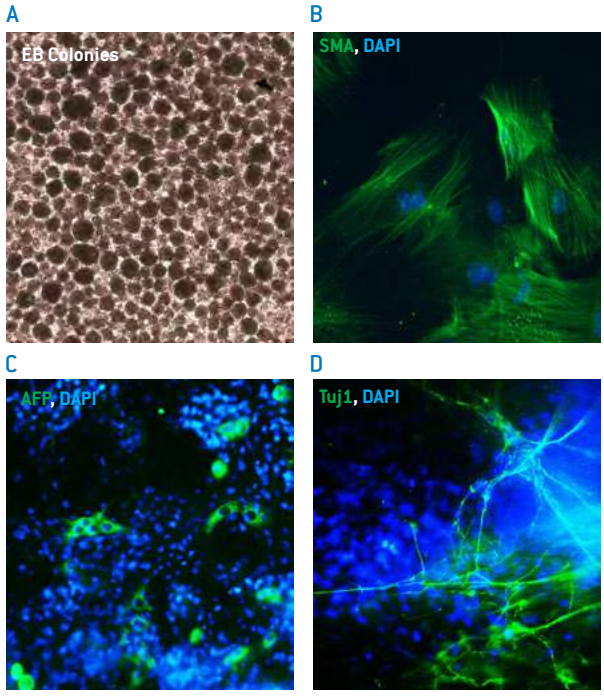
- Lonza Human Peripheral Blood Mononuclear Cells as positive control donor
- New L7™ PBMC Priming-Recovery Basal Medium for priming of PBMCs towards erythroblasts prior to reprogramming and optimal recovery post transfection
- New L7™ PBMC Reprogramming Enhancers A and B for optimal reprogramming results
- 4D-Nucleofector™ X Unit
- P3 Primary Cell 4D-Nucleofector™ Kit

Benefits

- Ready-to-use reprogramming protocol
- Seamless combination with our L7™ hPSC Culture System for feeder- and xeno-free iPSC culture

Applications

- Reprogramming of PBMCs using the 4D-Nucleofector™ System



***In vitro* differentiation of PBMC-derived iPSCs into embryoid bodies (EBs).** After 14 days of differentiation in the presence of FBS, differentiated PBMC derived hiPSCs were tested for the expression of proteins of representative cell types of the three germ layers. (A) EBs formed after differentiation of PBMC derived hiPSCs. (B) Mesoderm, stained with SMA (green) antibody targeting smooth muscle actin. (C) Endoderm stained with AFP (green) antibody against alpha-fetoprotein. (D) Ectoderm, stained with Tuj1 (green) antibody against neuron-specific beta-tubulin III.

Ordering Information – L7™ PBMC Reprogramming Bundles

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2702	CC-2702	hPBMC – Human Peripheral Blood Mononuclear Cells	Cryopreserved, volume discount available	≥50 million cells/vial
FP-5124	FP-5124	L7™ PBMC Priming-Recovery BulletKit	Containing: L7™ PBMC Priming-Recovery Basal Medium, L7™ PBMC Reprogramming Enhancers A and B	10 reactions
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions

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4D-Nucleofector™ System	175

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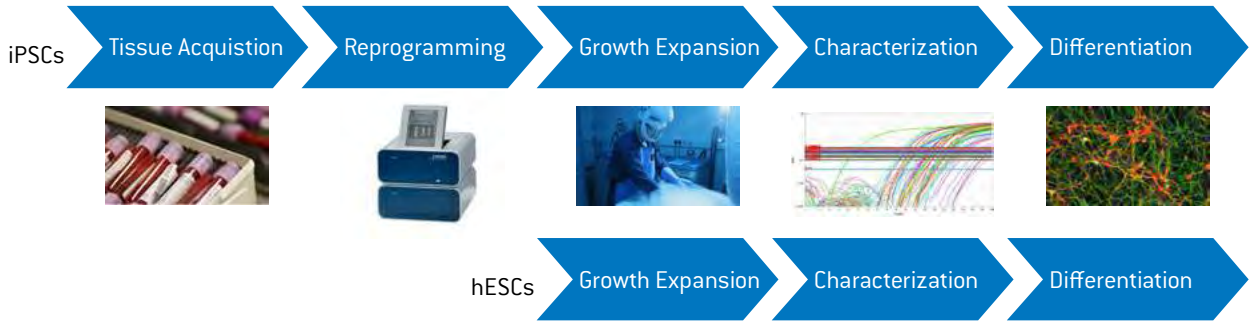
Pluripotent Stem Cell Services

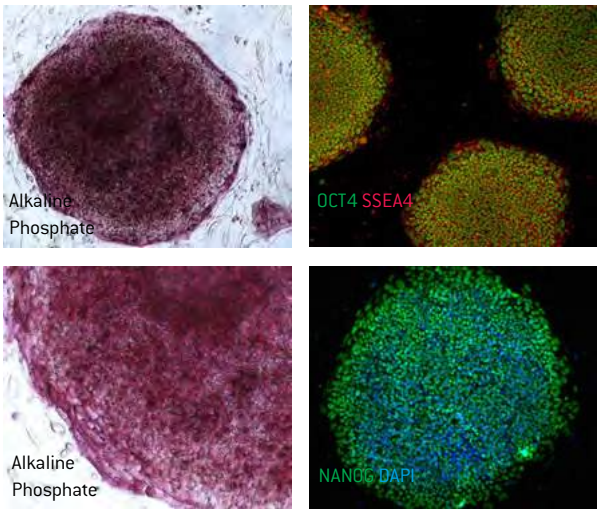
Lonza established a new strategic vision to become the leading supplier to the regenerative medicine industry. To realize this vision, Lonza created the Pluripotent Stem Cell Innovation Center. Pluripotent stem cells (PSCs) have the ability to generate any of the 220+ cell types in the human body. And because of this unique attribute, these cells have great potential in basic research, drug discovery and cell replacement therapies.



PSC Services Lonza has built up expertise, capacity, and capabilities in pluripotent stem cell research and their application to cGMP manufacturing. Researchers can now access this expertise through our PSC service offering from iPSC generation to process development and differentiation.

Our services span the full value chain of pluripotent stem cells from tissue acquisition to differentiation:

- **Tissue Acquisition** – We have a dedicated team that procures both research and cGMP grade tissue according to the highest ethical standards and in compliance with government regulations.
- **Reprogramming** – We offer cGMP and non-cGMP iPSC generation under feeder- and feeder-free conditions using a zero-footprint technology
- **Growth / Expansion / Banking** – We have established protocols using all of the common medium, matrix, and passaging methods. We also have the infrastructure and resources to support small- and large-scale culture and banking of PSCs.
- **Characterization** – We offer all the standard methods of characterizing PSCs including thawing efficiency, myoplasma and sterility testing, karyotype analysis, short tandem repeat genotyping, pluripotency marker expression (flow cytometry and immunofluorescence), and pluripotency assays (embryoid body and teratoma formation).
- **Differentiation** – We have established protocols for the production of PSC-derived motor neurons, dopaminergic neurons, and neural stem cells. We also have development programs underway to add to our differentiation portfolio of therapeutically relevant cell types
- **Process development** – Over the years we have built up expertise in the differentiation of high purity, functional cell types. Our team is well versed in technology transfer and optimization of manufacturing protocols



Human induced pluripotent stem cells express hESC-associated markers POU5F1/OCT4 (green) and SSEA4 (red) counterstained with DAPI (blue).

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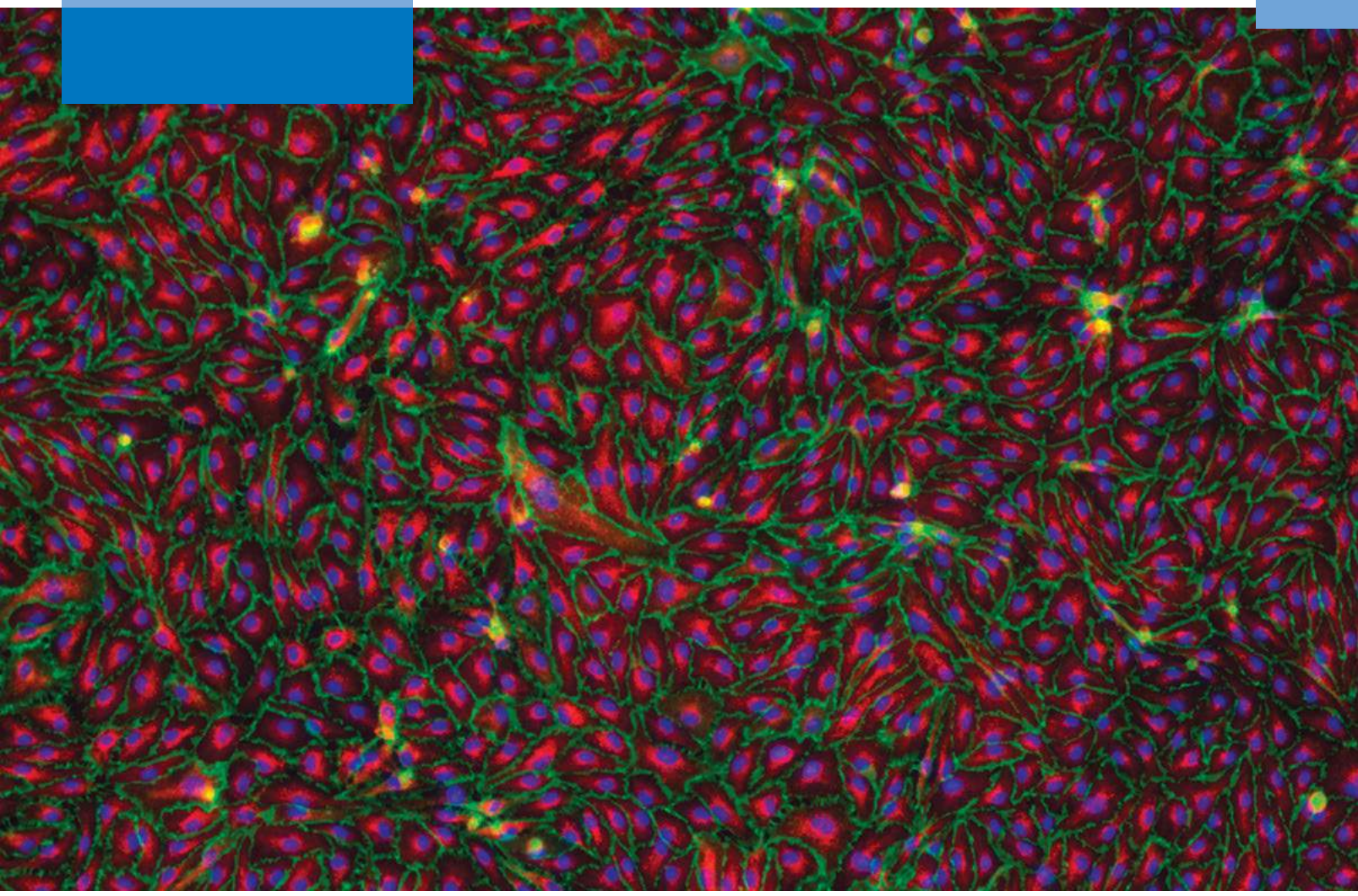
2 Primary Cells and Media



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These products are for research use only.
Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vivo* procedures.

WARNING: Clonetics™ AND Poietics™ products contain human source material, treat as potentially infectious.
Each donor , with the exception of Cord Blood derived products, is tested and found non-reactive by an FDA approved method for the presence of HIV-1, Hepatitis B Virus, and Hepatitis C Virus. Most, but not all, Cord Blood derived products are tested and found non-reactive by an FDA approved method for the presence of Hepatitis C Virus.
Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, Hepatitis B Virus, and Hepatitis C Virus. Testing cannot offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, "*Biosafety in Microbiological and Biomedical Laboratories*", 5th ed. If you require further information, please contact your site Safety Officer or Scientific Support.

Product Warranty – Cultures have a finite lifespan *in vitro*.
Lonza guarantees the performance of its cells up to two years from purchase only if appropriate Clonetics™ or Poietics™ Media and Reagents are used exclusively, and the recommended storage and use protocols are followed. Cell and media performance is not guaranteed if any modifications are made to the complete cell system.

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Quick Reference Guide

Cell Type / Tissue	Cell Cat. No.	Recommended Medium	Media Cat. No.	Page
Adventitial Fibroblasts				
Aorta	CC-7014	SCGM™	CC-3205	58
Astrocytes				
Human Brain	CC-2565	AGM™	CC-3186	71
C57 Mouse Brain – Mixed	M-AsM-330	AGM™	CC-3186	96
CD1 Mouse Brain – Mixed	M-AsM-430	AGM™	CC-3186	96
Rat Brain Cx-Hi-Cp – Mixed	R-AsM-530	AGM™	CC-3186	96
Rat Brain – Cortex	R-CxAs-520	AGM™	CC-3186	96
Rat Brain – Hippocampus	R-HiAs-521	AGM™	CC-3186	96
Rat Brain – Striatum	R-CpAs-522	AGM™	CC-3186	96
Bone				
Osteoblasts	CC-2538	OGM™	CC-3207	84
Osteoclast Precursors	2T-110	OCP	PT-8001	25
Rat Calvariae Osteoclast	R-OST-583	DMEM™	12-604F	98
Cardiac Myocytes				
Rat Cardiac Myocytes	R-CM-561	RCGM™	CC-4515	92
Chondrocytes				
Cartilage	CC-2550	CGM™	CC-3216	84
Dendritic Cells				
Blood	CC-2701	LGM™ 3	CC-3211	102
Endothelial Cells – Large Vessel				
Aorta	CC-2535	EGM™ 2	CC-3162	58,64
Aortic – Diabetes Type I	CC-2919	EGM™ 2	CC-3162	58,64
Aortic – Diabetes Type II	CC-2920	EGM™ 2	CC-3162	58,64
Coronary Artery	CC-2585	EGM™ 2MV	CC-3202	58,64
Coronary Artery – Diabetes Type I	CC-2921	EGM™ 2MV	CC-3202	58,64
Coronary Artery – Diabetes Type II	CC-2922	EGM™ 2MV	CC-3202	58,64
Iliac Artery	CC-2545	EGM™ 2MV	CC-3202	64
Pulmonary Artery	CC-2530	EGM™ 2	CC-3162	64,77
Umbilical Vein – Single Donor	C2517A	EGM™ 2	CC-3162	64
Umbilical Vein – Pooled Donor	C2519A	EGM™ 2	CC-3162	64
Endothelial Cells – Microvascular				
Bladder	CC-7016	EGM™ 2MV	CC-3202	55,66
Blood – Adult	CC-2811	EGM™ 2MV	CC-3202	61,66
Blood – Neonatal	CC-2813	EGM™ 2MV	CC-3202	61,66
Dermal – Adult	CC-2543	EGM™ 2MV	CC-3202	61,66
Dermal Adult – Diabetes Type I	CC-2929	EGM™ 2MV	CC-3202	61,66
Dermal Adult – Diabetes Type II	CC-2930	EGM™ 2MV	CC-3202	61,66
Dermal – Neonatal	CC-2505	EGM™ 2MV	CC-3202	61,66
Dermal – Neonatal, pooled	CC-2516	EGM™ 2MV	CC-3202	66
Cardiac	CC-7030	EGM™ 2MV	CC-3202	58,66
Cardiac – Diabetes Type I	CC-2927	EGM™ 2MV	CC-3202	58,66

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Cell Type / Tissue	Cell Cat. No.	Recommended Medium	Media Cat. No.	Page
Cardiac – Diabetes Type II	CC-2928	EGM™ 2MV	CC-3202	58,66
Lung	CC-2527	EGM™ 2MV	CC-3202	66,77
Lymphatic – Adult	CC-2810	EGM™ 2MV	CC-3202	66,69
Lymphatic – Neonatal	CC-2812	EGM™ 2MV	CC-3202	61,66,69
Uterus	CC-2564	EGM™ 2MV	CC-3202	66,83
Epithelial Cells				
Bronchial / Tracheal (with Retinoic Acid)	CC-2540S	B-ALI™	193514	77
Bronchial / Tracheal (with Retinoic Acid)	CC-2540	BEGM™	CC-3170	77
Bronchial / Tracheal (without Retinoic Acid)	CC-2541	BEGM™	CC-3170	77
Diseased Bronchial / Tracheal – Asthma	194911	BEGM™	CC-3170	78
Diseased Bronchial / Tracheal – Cystic Fibrosis	196979	BEGM™	CC-3170	78
Diseased Bronchial / Tracheal – COPD	195275	BEGM™	CC-3170	78
Kidney (Renal)	CC-2556	REGM™	CC-3190	80
Kidney (Renal) – Cortex	CC-2554	REGM™	CC-3190	80
Kidney (Renal) – Proximal Tubule	CC-2553	REGM™	CC-3190	80
Kidney (Renal) – Proximal Tubule – Diabetes Type II	CC-2925	REGM™	CC-3190	80
Mammary	CC-2551	MEGM™	CC-3150	70
Prostate	CC-2555	PrEGM™	CC-3166	74,83
Small Airway	CC-2547	SAGM™	CC-3118	77
Diseased Small Airway (Asthma)	CC-2932	SAGM™	CC-3118	78
Diseased Small Airway (COPD)	CC-2934	SAGM™	CC-3118	78
Diseased Small Airway (Cystic Fibrosis)	CC-2933	SAGM™	CC-3118	78
Fibroblasts				
Cardiac – Aortic	CC-2903	FGM™ 3	CC-4526	58
Cardiac – Ventricular	CC-2904	FGM™ 3	CC-4526	58
Dermal – Adult	CC-2511	FGM™ 2	CC-3132	61
Dermal – Neonatal	CC-2509	FGM™ 2	CC-3132	61
Diseased Lung (COPD)	195277	FGM™ 2	CC-3132	78
Diseased Lung (Asthma)	194912	FGM™ 2	CC-3132	78
Diseased Lung (Cystic Fibrosis)	194843	FGM™ 2	CC-3132	78
Embryonic – Mouse	M-FB-481	DMEM	12-604F	93
Intestinal Myofibroblasts	CC-2902	SmGM™ 2	CC-3182	68
Lung	CC-2512	FGM™ 2	CC-3132	77
Periodontal Ligament	CC-7049	SCGM™	CC-3205	84
Keratinocytes				
Epidermal Adult – Diabetes Type II	CC-2926	KGM™ Gold	192060	61
Epidermal Adult – Normal Human	192627	KGM™ Gold	192060	61
Epidermal – Neonatal	192907	KGM™ Gold	192060	61
Epidermal – Neonatal, pooled	192906	KGM™ Gold	192060	61
Melanocytes				
Neonatal	CC-2504	MGM™ 4	CC-3249	61
Adult	CC-2586	MGM™ 4	CC-3249	61

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Cell Type / Tissue	Cell Cat. No.	Recommended Medium	Media Cat. No.	Page
Mesangial Cells				
Kidney	CC-2559	MsGM™	CC-3146	80
Myoblasts				
Skeletal Muscle	CC-2580	SkGM™ 2	CC-3245	87
Skeletal Muscle Myoblasts – Diabetes Type I	CC-2900	SkGM™ 2	CC-3245	87
Skeletal Muscle Myoblasts – Diabetes Type II	CC-2901	SkGM™ 2	CC-3245	87
Preadipocytes Cells				
Subcutaneous	PT-5001	PGM™ 2	PT-8002	27
Subcutaneous– Diabetes Type I	PT-5021	PGM™ 2	PT-8002	27
Subcutaneous– Diabetes Type II	PT-5022	PGM™ 2	PT-8002	27
Visceral	PT-5005	PGM™ 2	PT-8002	27
Visceral– Diabetes Type I	PT-5023	PGM™ 2	PT-8002	27
Visceral– Diabetes Type II	PT-5024	PGM™ 2	PT-8002	27
Neural Progenitor Cells				
Brain	PT-2599	NPMM™	CC-3209	24
Neurons				
CD1 Mouse Brain – Cortex	M-Cx-400	PNGM™	CC-4461	96
CD1 Mouse Brain – Hippocampus	M-Hi-401	PNGM™	CC-4461	96
CD1 Mouse Brain – Striatum	M-Cp-402	PNGM™	CC-4461	96
C57 Mouse Brain – Cortex	M-Cx-300	PNGM™	CC-4461	96
C57 Mouse Brain – Striatum	M-Cp-302	PNGM™	CC-4461	96
Rat Brain – Cortex	R-Cx-500	PNGM™	CC-4461	96
Rat Brain – Striatum	R-Cp-502	PNGM™	CC-4461	96
Rat Brain – Hippocampus	R-Hi-501	PNGM™	CC-4461	96
Rat Brain – Hypothalamus	R-Hth-507	PNGM™	CC-4461	96
Rat Brain Cerebellum – Granule Cells	R-Cb-503	PNGM™ A	CC-4512	96
Rat Embryo – Dorsal Root Ganglion	R-eDRG-515	PNGM™	CC-4461	96
Rat Retinal	R-ReT-508	PNGM™	CC-4461	97
Rat Spinal Cord – Dorsal Root Ganglion	R-Drg-505	PNGM™	CC-4461	96
Stem Cells				
Bone Marrow	PT-2501	MSCGM™	PT-3001	29
Dental Pulp	PT-5025	DPSC-GM	PT-3005	26
Adipose	PT-5006	ADSC-GM	PT-4505	19
Adipose - Diabetes Type I	PT-5007	ADSC-GM	PT-4505	19
Adipose - Diabetes Type II	PT-5008	ADSC-GM	PT-4505	19
Skeletal Muscle Cells				
Skeletal Muscle	CC-2561	SkGM™	CC-3160	87

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Part Number	Proliferating Cell Type Description	Recommended Media	6-well	12-well	24-well	48-well	96-well	T-25	T-75	T-150	T-225
Proliferating Cells – Normal											
CC-7014	AoAF – Human Aortic Adventitial Fibroblasts	SCGM™ BulletKit™	CC-7014W6	CC-7014W12	CC-7014W24	CC-7014W48	CC-7014W96	CC7014T25	CC7014T75	CC7014T150	CC7014T225
CC-2571	AoSMC – Human Aortic Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2571W6	CC-2571W12	CC-2571W24	CC-2571W48	CC-0148	CC-2671	CC-0234	CC2571T150	CC2571T225
CC-2533	BdSMC – Human Bladder Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2533W6	CC-2533W12	CC-2533W24	CC-2533W48	CC-2533W96	CC-2533T25	CC-2533T75	CC2533T150	CC2533T225
CC-2576	BSMC – Human Bronchial Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2576W6	CC-2576W12	CC-2576W24	CC-2576W48	CC-0180	CC-2676	CC-0240	CC2576T150	CC2576T225
CC-2583	CASMC – Human Coronary Artery Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2583W6	CC-2583W12	CC-2583W24	CC-2583W48	CC-0096	CC-2683	CC-0258	CC2583T150	CC2583T225
CC-2535	HAEC – Human Aortic Endothelial Cells	EGM™ 2 BulletKit™	CC-2535W6	CC-2535W12	CC-2535W24	CC-2535W48	CC-0132	CC-2635	CC-0222	CC2535T150	CC2535T225
CC-2585	HCAEC – Human Coronary Artery Endothelial Cells	EGM™ 2MV BulletKit™	CC-2585W6	CC-2585W12	CC-2585W24	CC-2585W48	CC-0188	CC-2685	CC-0261	CC2585T150	CC2585T225
CC-2545	HIAEC – Human Iliac Artery Endothelial Cells	EGM™ 2MV BulletKit™	CC-2545W6	CC-2545W12	CC-2545W24	CC-2545W48	CC-0095	CC-2645	CC-0291	CC2545T150	CC2545T225
CC-2551	HMEC – Human Mammary Epithelial Cells	MEGM™ BulletKit™	CC-2551W6	CC-2551W12	CC-2551W24	CC-2551W48	CC-0140	CC-2651	CC-0228	CC2551T150	CC2551T225
CC-7016	HMVEC-Bd – Human Bladder Microvascular Endothelial Cells	EGM™ 2MV BulletKit™	CC-7016W6	CC-7016W12	CC-7016W24	CC-7016W48	CC-7016W96	CC-7016T25	CC-7016T75	CC7016T150	CC7016T225
CC-7030	HMVEC-C – Human Cardiac Microvascular Endothelial Cells	EGM™ 2MV BulletKit™	CC-7030W6	CC-7030W12	CC-7030W24	CC-7030W48	CC-7030W96	CC-7030T25	CC-7030T75	CC-7030T150	CC-7030T225
CC-2543	HMVEC-dAd – Human Dermal Microvascular Endothelial Cells – Adult	EGM™ 2MV BulletKit™	CC-2543W6	CC-2543W12	CC-2543W24	CC-2543W48	CC-2543W96	CC-2643	CC-0207	CC2543T150	CC2543T225
CC-2811	HMVEC-dBlAd – Human Dermal Blood Microvascular Endothelial Cells – Adult	EGM™ 2MV BulletKit™	CC-2811W6	CC-2811W12	CC-2811W24	CC-2811W48	CC-2811W96	CC-2811T25	CC-2811T75	CC-2811T150	CC-2811T225
CC-2813	HMVEC-dBlNeo – Human Dermal Blood Microvascular Endothelial Cells – Neonatal	EGM™ 2MV BulletKit™	CC-2813W6	CC-2813W12	CC-2813W24	CC-2813W48	CC-2813W96	CC-2813T25	CC-2813T75	CC-2813T150	CC-2813T225
CC-2810	HMVEC-dLyAd – Human Dermal Lymphatic Microvascular Endothelial Cells – Adult	EGM™ 2MV BulletKit™	CC-2810W6	CC-2810W12	CC-2810W24	CC-2810W48	CC-2810W96	CC-2810T25	CC-2810T75	CC-2810T150	CC-2810T225
CC-2812	HMVEC-dLyNeo – Human Dermal Lymphatic Microvascular Endothelial Cells – Neonatal	EGM™ 2MV BulletKit™	CC-2812W6	CC-2812W12	CC-2812W24	CC-2812W48	CC-2812W96	CC-2812T25	CC-2812T75	CC-2812T150	CC-2812T225
CC-2516	HMVEC-dNeo – Human Dermal Microvascular Endothelial Cells – Neonatal, Pooled	EGM™ 2MV BulletKit™	CC-2516W6	CC-2516W12	CC-2516W24	CC-2516W48	CC-2516W96	CC-2616	CC-0288	CC2516T150	CC2516T225
CC-2505	HMVEC-dNeo – Human Dermal Microvascular Endothelial Cells – Neonatal, Single Donor	EGM™ 2MV BulletKit™	CC-2505W6	CC-2505W12	CC-2505W24	CC-2505W48	CC-0112	CC-2605	CC-0246	CC2505T150	CC2505T225
CC-2527	HMVEC-L – Human Lung Microvascular Endothelial Cells	EGM™ 2MV BulletKit™	CC-2527W6	CC-2527W12	CC-2527W24	CC-2527W48	CC-0184	CC-2627	CC-0264	CC2527T150	CC2527T225
CC-2814	HMVEC-Lly – Human Lung Lymphatic Microvascular Endothelial Cells	EGM™ 2MV BulletKit™	CC-2814W6	CC-2814W12	CC-2814W24	CC-2814W48	CC-2814W96	CC-2814T25	CC-2814T75	CC-2814T150	CC-2814T225
CC-2530	HPAEC – Human Pulmonary Artery Endothelial Cells	EGM™ 2 BulletKit™	CC-2530W6	CC-2530W12	CC-2530W24	CC-2530W48	CC-0128	CC-2630	CC-0219	CC2530T150	CC2530T225
CC-7049	HPdLF – Human Periodontal Ligament Fibroblasts	SCGM™ BulletKit™	CC-7049W6	CC-7049W12	CC-7049W24	CC-7049W48	CC-7049W96	CC-7049T25	CC-7049T75	CC-7049T150	CC-7049T225
CC-2554	HRCE – Human Renal Cortical Epithelial Cells	REGM™ BulletKit™	CC-2554W6	CC-2554W12	CC-2554W24	CC-2554W48	CC-0172	CC-2654	CC-0270	CC2554T150	CC2554T225
CC-2556	HRE – Human Renal Epithelial Cells	REGM™ BulletKit™	CC-2556W6	CC-2556W12	CC-2556W24	CC-2556W48	CC-2556W96	CC-2556T25	CC-2556T75	CC2556T150	CC2556T225
CC-2580	HSMM – Human Skeletal Muscle Myoblasts	SkGM™ 2 BulletKit™	CC-2580W6	CC-2580W12	CC-2580W24	CC-2580W48	CC-2580W96	CC-2580T25	CC-2580T75	CC2580T150	CC2580T225
CC-2519	HUVEC – Human Umbilical Vein Endothelial Cells, Pooled	EGM™ BulletKit™	CC-2519W6	CC-2519W12	CC-2519W24	CC-2519W48	CC-2519W96	CC-2619	CC-0276	CC2519T150	CC2519T225
C2519A	HUVEC – Human Umbilical Vein Endothelial Cells, Pooled	EGM™ 2 BulletKit™	C2519AW6	C2519AW12	C2519AW24	C2519AW48	C2519AW96	C2519AT25	C2519AT75	C2519AT150	C2519AT225
C2517AS	HUVEC – Human Umbilical Vein Endothelial Cells, Pooled, S-Part	EGM™ 2 BulletKit™	C2519ASW6	C2519ASW12	C2519ASW24	C2519ASW48	C2517ASW96	C2519AST25	C2519AST75	C2519AST150	C2519AST225
CC-2517	HUVEC – Human Umbilical Vein Endothelial Cells, Single Donor	EGM™ BulletKit™	CC-2517W6	CC-2517W12	CC-2517W24	CC-2517W48	CC-0124	CC-2617	CC-0216	CC2517T150	CC2517T225
C2517A	HUVEC – Human Umbilical Vein Endothelial Cells, Single Donor	EGM™ 2 BulletKit™	C2517AW6	C2517AW12	C2517AW24	C2517AW48	C2517AW96	C2517AT25	C2517AT75	C2517AT150	C2517AT225
C2517AS	HUVEC – Human Umbilical Vein Endothelial Cells, Single Donor, S-part	EGM™ 2 BulletKit™	C2517ASW6	C2517ASW12	C2517ASW24	C2517ASW48	C2517ASW96	C2517AST25	C2517AST75	C2517AST150	C2517AST225
CC-2550	NHAC-kn – Human Articular Chondrocytes	CGM™ BulletKit™	CC-2550W6	CC-2550W12	CC-2550W24	CC-2550W48	CC-2550W96	CC-2550T25	CC-2550T75	CC2550T150	CC2550T225
CC-2565	NHA – Human Astrocytes	AGM™ BulletKit™	CC-2565W6	CC-2565W12	CC-2565W24	CC-2565W48	CC-0093	CC-2665	CC-0297	CC2565T150	CC2565T225
CC-2540	NHBE – Human Bronchial /Tracheal Epithelial Cells	BEGM™ BulletKit™	CC-2540W6	CC-2540W12	CC-2540W24	CC-2540W48	CC-0136	CC-2640	CC-0225	CC2540T150	CC2540T225
CC-2541	NHBE – Human Bronchial /Tracheal Epithelial Cells	BEGM™ BulletKit™	CC-2541W6	CC-2541W12	CC-2541W24	CC-2541W48	CC-0100	CC-2641	CC-0285	CC2541T150	CC2541T225
CC-2511	NHDF-Ad – Human Dermal Fibroblasts – Adult	FGM™ 2 BulletKit™	CC-2511W48	CC-0160	CC-2511W12	CC-2511W24	CC-2511W6	CC-2611	CC-0252	CC2511T150	CC2511T225
CC-2509	NHDF-Neo – Human Dermal Fibroblasts – Neonatal	FGM™ 2 BulletKit™	CC-2509W6	CC-2509W12	CC-2509W24	CC-2509W48	CC-0116	CC-2609	CC-0210	CC2509T150	CC2509T225
CC-2501	NHEK-Ad – Human Epidermal Keratinocytes – Adult	KGM™ Gold BulletKit™	CC-2501W6	CC-2501W12	CC-2501W24	CC-2501W48	CC-0104	CC-2601	CC-0201	CC2501T150	CC2501T225
192627	NHEK-Ad – Normal Human Epidermal Kerationocytoes – Adult	KGM™ Gold BulletKit™	00192627W6	192627W12	192627W24	192627W48	192627W96	192627T25	192627T75	192627T150	192627T225
CC-2503	NHEK-Neo – Normal Human Epidermal Keratinocytes – Neonatal	KGM™ BulletKit™	CC-2503W6	CC-2503W12	CC-2503W24	CC-2503W48	CC-0108	CC-2603	CC-0204	CC2503T150	CC2503T225
192907	NHEK-Neo – Normal Human Epidermal Keratinocytes – Neonatal	KGM™ Gold BulletKit™	192907W6	192907W12	192907W24	192907W48	192907W96	192907T25	192907T75	192907T150	192907T225
CC-2507	NHEK-Neo – Normal Human Epidermal Keratinocytes – Neonatal, Pooled	KGM™ BulletKit™	CC-2507W6	CC-2507W12	CC-2507W24	CC-2507W48	CC-0156	CC-2607	CC-0255	CC2507T150	CC2507T225
192906	NHEK-Neo – Normal Human Epidermal Keratinocytes – Neonatal, Pooled	KGM™ Gold BulletKit™	192906W6	192906W12	192906W24	192906W48	192906W96	192906T25	192906T75	192906T150	192906T225
CC-2504	NHEM-Neo – Normal Human Epidermal Melanocytes – Neonatal	MGM™ 4 BulletKit™	CC-2504W6	CC-2504W12	CC-2504W24	CC-2504W48	CC-2504W96	CC-2504T25	CC-2504T75	CC-2504T150	CC-2504T225
CC-2512	NHLF – Normal Human Lung Fibroblasts	FGM™ 2 BulletKit™	CC-2512W6	CC-2512W12	CC-2512W24	CC-2512W48	CC2512T150	CC-2612	CC-0282	CC2512T225	CC-0164
CC-2559	NHMC – Normal Human Mesangial Cells	MsGM™ BulletKit™	CC-2559W6	CC-2559W12	CC-2559W24	CC-2559W48	CC-0176	CC-2659	CC-0273	CC2559T150	CC2559T225

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Part Number	Proliferating Cell Type Description	Recommended Media	6-well	12-well	24-well	48-well	96-well	T-25	T-75	T-150	T-225
Proliferating Cells – Normal											
CC-2538	NHOst – Normal Human Osteoblasts	OGM™ BulletKit™	CC-2538W6	CC-2538W12	CC-2538W24	CC-2538W48	CC-2538W96	CC-2538T25	CC-2538T75	CC2538T150	CC2538T225
CC-2581	PASMC – Human Pulmonary Artery Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2581W6	CC-2581W12	CC-2581W24	CC-2581W48	CC-0152	CC-2681	CC-0237	CC2581T150	CC2581T225
CC-2555	PrEC – Human Prostate Epithelial Cells	PrEGM™ BulletKit™	CC-2555W6	CC-2555W12	CC-2555W24	CC-2555W48	CC-0088	CC-2655	CC-0310	CC2555T150	CC2555T225
CC-2508	PrSC – Human Prostate Stromal Cells	SCGM™ BulletKit™	CC-2508W6	CC-2508W12	CC-2508W24	CC-2508W48	CC-2508W96	CC-2608	CC-2508T75	CC2508T150	CC2508T225
CC-2587	PrSMC – Human Prostate Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2587W6	CC-2587W12	CC-2587W24	CC-2587W48	CC-2587W96	CC-2587T25	CC-2587T75	CC2587T150	CC2587T225
CC-2553	RPTEC – Human Renal Proximal Tubule Cells	REGM™ BulletKit™	CC-2553W6	CC-2553W12	CC-2553W24	CC-2553W48	CC-0168	CC-2653	CC-0267	CC2553T150	CC2553T225
CC-2547	SAEC – Human Small Airway Epithelial Cells	SAGM™ BulletKit™	CC-2547W6	CC-2547W12	CC-2547W24	CC-2547W48	CC-0094	CC-2647	CC-0294	CC2547T150	CC2547T225
CC-2561	SkMC – Human Skeletal Muscle Cells	SkGM™ BulletKit™	CC-2561W6	CC-2561W12	CC-2561W24	CC-2561W48	CC-0144	CC-2661	CC-0231	CC2561T150	CC2561T225
CC-2579	UASMC – Human Umbilical Artery Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2579W6	CC-2579W12	CC-2579W24	CC-2579W48	CC-0192	CC-2679	CC-0243	CC2579T150	CC2579T225
CC-2564	UtMVEC-Myo – Human Uterine Microvascular Endothelial Cells	EGM™ 2MV BulletKit™	C2564AW6	C2564AW12	C2564AW24	C2564AW48	C2564AW96	C2564AT25	C2564AT75	C2564AT150	C2564AT225
CC-2562	UtSMC – Human Uterine Smooth Muscle Cells	SmGM™ 2 BulletKit™	CC-2562W6	CC-2562W12	CC-2562W24	CC-2562W48	CC-0089	CC-2662	CC-0313	CC2562T150	CC2562T225
194987	H-RPE – Human Retinal Pigment Epithelial Cells	RtEGM™ BulletKit™	194987W6	194987W12	194987W24	194987W48	194987W96	194987T25	194987T75	194987T150	194987T225
CC-2586	NHEM-Ad – Normal Human Melanocytes – Adult	MGM™ 4 BulletKit™	CC-2586W6	CC-2586W12	CC-2586W24	CC-2586W48	CC-2586W96	CC-2586T25	CC-2586T75	CC-2586T150	CC-2586T225
CC-2902	InMyoFib – Intestinal Myofibroblasts	SmGM™ 2 BulletKit™	CC-2902W6	CC-2902W12	CC-2902W24	CC-2902W48	CC-2902W96	CC-2902T25	CC-2902T75	CC-2902T150	CC-2902T225
CC-2903	NHCF-A – Normal Human Cardiac Fibroblasts – Atrial	FGM™ 3 BulletKit™	CC-2903W6	CC-2903W12	CC-2903W24	CC-2903W48	CC-2903W96	CC-2903T25	CC-2903T75	CC-2903T150	CC-2903T225
CC-2904	NHCF-V – Normal Human Cardiac Fibroblasts – Ventricular	FGM™ 3 BulletKit™	CC-2904W6	CC-2904W12	CC-2904W24	CC-2904W48	CC-2904W96	CC-2904T25	CC-2904T75	CC-2904T150	CC-2904T225

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Part Number	Proliferating Cell Type Description	Recommended Media	6-well	12-well	24-well	48-well	96-well	T-25	T-75	T-150	T-225
Proliferating Cells – Diseased											
194843	D-HLF-CF – Diseased Human Lung Fibroblasts – Cystic Fibrosis	FGM™ 2 BulletKit™	194843W6	194843W12	194843W24	194843W48	194843W96	194843T25	194843T75	194843T150	194843T225
194850	D-BSMC-As – Diseased Bronchial Smooth Muscle Cells – Asthma	SmGM™ 2 BulletKit™	194850W6	194850W12	194850W24	194850W48	194850W96	194850T25	194850T75	194850T150	194850T225
194911	D-HBE-As – Diseased Human Bronchial/Tracheal Epithelial Cells – Asthma	BEGM™ BulletKit™	194911W6	194911W12	194911W24	194911W48	194911W96	194911T25	194911T75	194911T150	194911T225
194912	D-HLF-As – Diseased Human Lung Fibroblast Cells – Asthma	FGM™ 2 BulletKit™	194912W6	194912W12	194912W24	194912W48	194912W96	194912T25	194912T75	194912T150	194912T225
195274	D-BSMC-COPD – Diseased Bronchial Smooth Muscle Cells – COPD	SmGM™ 2 BulletKit™	195274W6	195274W12	195274W24	195274W48	195274W96	195274T25	195274T75	195274T150	195274T225
195275	D-HBE-COPD – Diseased Human Bronchial/Tracheal Epithelial Cells – COPD	BEGM™ BulletKit™	195275W6	195275W12	195275W24	195275W48	195275W96	195275T25	195275T75	195275T150	195275T225
195277	D-HLF-COPD – Diseased Human Lung Fibroblast Cell – COPD	FGM™ 2 BulletKit™	195277W6	195277W12	195277W24	195277W48	195277W96	195277T25	195277T75	195277T150	195277T225
196979	D-HBEC-CF – Diseased Human Bronchial/Tracheal Epithelial Cells – Cystic Fibrosis	BEGM™ BulletKit™	196979W6	196979W12	196979W24	196979W48	196979W96	196979T25	196979T75	196979T150	196979T225
196980	D-HBSMC-CF – Diseased Human Bronchial Smooth Muscle Cells – Cystic Fibrosis	SmGM™ 2 BulletKit™	196980W6	196980W12	196980W24	196980W48	196980W96	196980T25	196980T75	196980T150	196980T225
CC-2900	D-HSMM – Diseased Human Skeletal Muscle Myoblasts – Diabetes Type I	SkGM™ 2 BulletKit™	CC-2900W6	CC-2900W12	CC-2900W24	CC-2900W48	CC-2900W96	CC-2900T25	CC-2900T75	CC-2900T150	CC-2900T225
CC-2901	D-HSMM – Diseased Human Skeletal Muscle Myoblasts – Diabetes Type II	SkGM™ 2 BulletKit™	CC-2901W6	CC-2901W12	CC-2901W24	CC-2901W48	CC-2901W96	CC-2901T25	CC-2901T75	CC-2901T150	CC-2901T225
CC-2913	D-PASMC – Diseased Human Pulmonary Artery Smooth Muscle – Diabetes Type II	SmGM™ 2 BulletKit™	CC-2913W6	CC-2913W12	CC-2913W24	CC-2913W48	CC-2913W96	CC-2913T25	CC-2913T75	CC-2913T150	CC-2913T225
CC-2914	D-AoSMC – Diseased Human Aortic Smooth Muscle – Diabetes Type I	SmGM™ 2 BulletKit™	CC-2914W6	CC-2914W12	CC-2914W24	CC-2914W48	CC-2914W96	CC-2914T25	CC-2914T75	CC-2914T150	CC-2914T225
CC-2915	D-PASMC – Diseased Human Pulmonary Artery Smooth Muscle Cells – Diabetes Type I	SmGM™ 2 BulletKit™	CC-2915W6	CC-2915W12	CC-2915W24	CC-2915W48	CC-2915W96	CC-2915T25	CC-2915T75	CC-2915T150	CC-2915T225
CC-2916	D-AoSMC – Diseased Human Aortic Smooth Muscle – Diabetes Type II	SmGM™ 2 BulletKit™	CC-2916W6	CC-2916W12	CC-2916W24	CC-2916W48	CC-2916W96	CC-2916T25	CC-2916T75	CC-2916T150	CC-2916T225
CC-2917	D-CASMC – Diseased Human Coronary Artery Smooth Muscle – Diabetes Type I	SmGM™ 2 BulletKit™	CC-2917W6	CC-2917W12	CC-2917W24	CC-2917W48	CC-2917W96	CC-2917T25	CC-2917T75	CC-2917T150	CC-2917T225
CC-2918	D-CASMC – Diseased Human Coronary Artery Smooth Muscle – Diabetes Type II	SmGM™ 2 BulletKit™	CC-2918W6	CC-2918W12	CC-2918W24	CC-2918W48	CC-2918W96	CC-2918T25	CC-2918T75	CC-2918T150	CC-2918T225
CC-2919	D-HAEC – Diseased Human Aortic Endothelial – Diabetes Type I	EGM™ 2 BulletKit™	CC-2919W6	CC-2919W12	CC-2919W24	CC-2919W48	CC-2919W96	CC-2919T25	CC-2919T75	CC-2919T150	CC-2919T225
CC-2920	D-HAEC – Diseased Human Aortic Endothelial – Diabetes Type II	EGM™ 2 BulletKit™	CC-2920W6	CC-2920W12	CC-2920W24	CC-2920W48	CC-2920W96	CC-2920T25	CC-2920T75	CC-2920T150	CC-2920T225
CC-2921	D-HCAEC – Diseased Human Coronary Artery Endothelial Cells – Diabetes Type I	EGM™ 2MV BulletKit™	CC-2921W6	CC-2921W12	CC-2921W24	CC-2921W48	CC-2921W96	CC-2921T25	CC-2921T75	CC-2921T150	CC-2921T225
CC-2922	D-HCAEC – Diseased Human Coronary Artery Endothelial – Diabetes Type II	EGM™ 2MV BulletKit™	CC-2922W6	CC-2922W12	CC-2922W24	CC-2922W48	CC-2922W96	CC-2922T25	CC-2922T75	CC-2922T150	CC-2922T225
CC-2923	D-HPAEC – Diseased Human Pulmonary Artery Endothelial Cells – Diabetes Type I	EGM™ 2 BulletKit™	CC-2923W6	CC-2923W12	CC-2923W24	CC-2923W48	CC-2923W96	CC-2923T25	CC-2923T75	CC-2923T150	CC-2923T225
CC-2924	D-HPAEC – Diseased Human Pulmonary Artery Endothelial Cells – Diabetes Type II	EGM™ 2 BulletKit™	CC-2924W6	CC-2924W12	CC-2924W24	CC-2924W48	CC-2924W96	CC-2924T25	CC-2924T75	CC-2924T150	CC-2924T225
CC-2925	D-RPTEC – Diseased Human Renal Proximal Tubule Epithelial Cells – Diabetes Type II	REGM™ BulletKit™	CC-2925W6	CC-2925W12	CC-2925W24	CC-2925W48	CC-2925W96	CC-2925T25	CC-2925T75	CC-2925T150	CC-2925T225
CC-2926	D-HEK-Ad – Diseased Human Adult Epidermal Keratinocytes – Diabetes Type II	KGM™ Gold BulletKit™	CC-2926W6	CC-2926W12	CC-2926W24	CC-2926W48	CC-2926W96	CC-2926T25	CC-2926T75	CC-2926T150	CC-2926T225
CC-2927	D-HMVEC – Diseased Cardiac Microvascular Endothelial Cells – Diabetes Type I	EGM™ 2MV BulletKit™	CC-2927W6	CC-2927W12	CC-2927W24	CC-2927W48	CC-2927W96	CC-2927T25	CC-2927T75	CC-2927T150	CC-2927T225
CC-2928	D-HMVEC – Diseased Cardiac Microvascular Endothelial Cells – Diabetes Type II	EGM™ 2MV BulletKit™	CC-2928W6	CC-2928W12	CC-2928W24	CC-2928W48	CC-2928W96	CC-2928T25	CC-2928T75	CC-2928T150	CC-2928T225
CC-2929	D-HMVEC – Diseased Human Dermal Microvascular Endothelial Cells – Diabetes Type I	EGM™ 2MV BulletKit™	CC-2929W6	CC-2929W12	CC-2929W24	CC-2929W48	CC-2929W96	CC-2929T25	CC-2929T75	CC-2929T150	CC-2929T225
CC-2930	D-HMVEC – Diseased Human Dermal Microvascular Endothelial Cells – Diabetes Type II	EGM™ 2MV BulletKit™	CC-2930W6	CC-2930W12	CC-2930W24	CC-2930W48	CC-2930W96	CC-2930T25	CC-2930T75	CC-2930T150	CC-2930T225
CC-2932	D-SAEC-As – Diseased Small Airway Epithelial Cells – Asthma	BEGM™ BulletKit™	CC-2932W6	CC-2932W12	CC-2932W24	CC-2932W48	CC-2932W96	CC-2932T25	CC-2932T75	CC-2932T150	CC-2932T225
CC-2933	D-SAEC – Diseased Small Airway Epithelial Cells – Cystic Fibrosis	BEGM™ BulletKit™	CC-2933W6	CC-2933W12	CC-2933W24	CC-2933W48	CC-2933W96	CC-2933T25	CC-2933T75	CC-2933T150	CC-2933T225
CC-2934	D-SAEC – Diseased Small Airway Epithelial Cells – COPD	BEGM™ BulletKit™	CC-2934W6	CC-2934W12	CC-2934W24	CC-2934W48	CC-2934W96	CC-2934T25	CC-2934T75	CC-2934T150	CC-2934T225

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Quick Reference Guide
Continued

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Primary Cells and Media / Quick Reference Guide

Europe – Customer Service: **+32 87 321 611**; order.europe@lonza.com; Scientific Support: **+32 87 321 611**; scientific.support.eu@lonza.com
International – Customer Service: **+1 301 898 7025**; Scientific Support: **scientific.support@lonza.com**

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Smooth Muscle Cells

Aorta	CC-2571	SmGM™ 2	CC-3182	58
Aorta – Diabetes Type I	CC-2914	SmGM™ 2	CC-3182	58
Aorta – Diabetes Type II	CC-2916	SmGM™ 2	CC-3182	58
Aorta – Rat	R-ASM-580	DMEM:F12	BE04-687Q	92
Bladder	CC-2533	SmGM™ 2	CC-3182	55
Bronchial	CC-2576	SmGM™ 2	CC-3182	77
Coronary Artery	CC-2583	SmGM™ 2	CC-3182	58
Coronary Artery– Diabetes Type I	CC-2917	SmGM™ 2	CC-3182	58
Coronary Artery– Diabetes Type II	CC-2918	SmGM™ 2	CC-3182	58
Diseased Bronchial (Asthma)	194850	SmGM™ 2	CC-3182	78
Diseased Bronchial (COPD)	195274	SmGM™ 2	CC-3182	78
Diseased Bronchial (Cystic Fibrosis)	196980	SmGM™ 2	CC-3182	78
Prostate	CC-2587	SmGM™ 2	CC-3182	74,83
Pulmonary Artery	CC-2581	SmGM™ 2	CC-3182	77
Pulmonary Artery– Diabetes Type I	CC-2915	SmGM™ 2	CC-3182	58
Pulmonary Artery– Diabetes Type II	CC-2913	SmGM™ 2	CC-3182	58
Umbilical Artery	CC-2579	SmGM™ 2	CC-3182	83
Uterus	CC-2562	SmGM™ 2	CC-3182	83

Stromal Cells

Prostate	CC-2508	SCGM™	CC-3205	74,83

Cell Type – Tissue	Source – Bone Marrow	Cord Blood	Peripheral	Page
Hematopoietic Cells				
Fresh Bone Marrow	1M-125			20
CD14⁺ Monocytes			2W-400A	103
CD34⁺ Cells	2M-101	2C-101		22
Mononuclear Cells	2M-125A		CC-2702	102
Stromal Cells	2M-302			20

Products are available in various sizes. Please refer to the catalog for size information.

Cat. No.	Description	Size	Page
Hematopoietic Cell Media			
PT-3926	HPGM™ Hematopoietic Progenitor Growth Medium	500 mL	23
CC-3211	LGM™ 3 Lymphocyte Growth Medium-3	500 mL	102-104
04-380Q	X-VIVO™ 10 Serum-free Hematopoietic Cell Medium – Chemically Defined With L-Glutamine, gentamicin, and phenol red	1 L	126
04-743Q	X-VIVO™ 10 Serum-free Hematopoietic Cell Medium – Chemically Defined With L-Glutamine, without gentamicin or phenol red	1 L	126
04-744Q	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined With L-Glutamine, without gentamicin or phenol red	1 L	126
BE02-060F	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically DefinedWith L-Glutamine, gentamicin, and phenol red	500 mL	126
04-418Q	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined With L-Glutamine, gentamicin, and phenol red	1 L	126
04-448Q	X-VIVO™ 20 Serum-free Hematopoietic Cell Medium – Chemically Defined With L-Glutamine, gentamicin and phenol red	1 L	126

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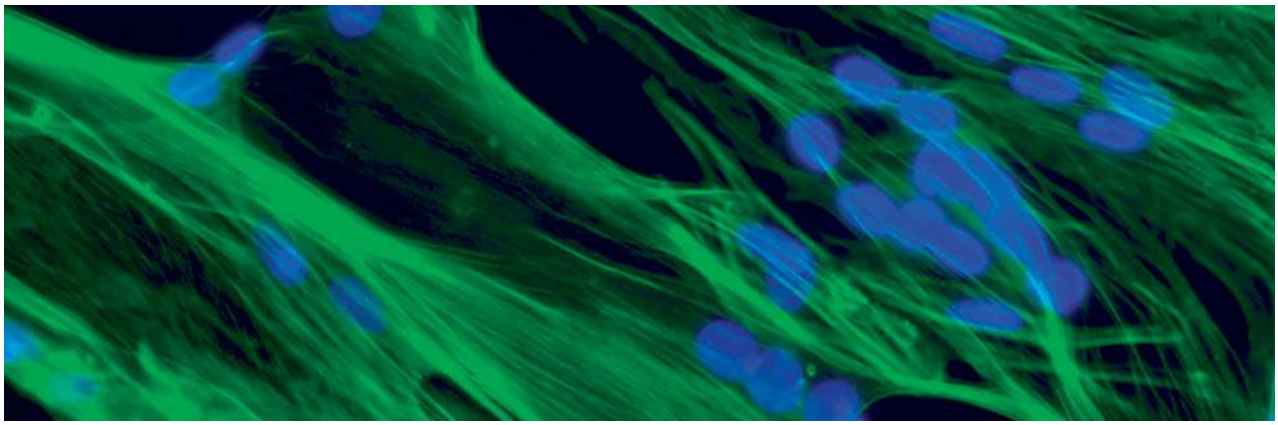
Clonetics™ Human Primary Cells and Media

In vivo relevance. *In vitro* results.



Clonetics™ Human Primary Cells and Media

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Introduction

Clonetics™ Human Primary Cells and Media include cells that are derived from normal and some diseased human tissues and test negative for HIV-1, Hepatitis B and C, mycoplasma and sterility. Immuno and special staining protocols, as well as characteristic morphology are used to characterize the cells and authenticate their identity. A Certificate of Analysis is available for each cryopreserved cell type and lot. The cells perform when used with the optimized system comprised of Clonetics™ Cells, Media, Reagents, and Protocol. Most cells are available cryopreserved, proliferating (in either flasks or plates), or as pellets in RNALater®, a reagent that inactivates RNases and stabilizes RNA within unfrozen tissues and cells.

Clonetics™ Media Kits have been specially designed to support the growth of these cells. These Media BulletKits™ are comprised of basal media and SingleQuots™ Kits of growth factors and supplements. For detailed information about these media systems, please See pages 400–409.

General Cell and Media Information

- Proliferating cells are offered in the following formats, Flasks (T-25, T-75, T-150, T-225) and Multiwell Plates (6, 12, 24, 48, and 96-wells). Contact Customer Service for order placement and delivery schedules, or Scientific Support for any other questions regarding alternative formats for cell culture reagents
- Cell pellets in RNALater® are available as well with 10 million cells/pellet, contact Customer Service for order placement
- Clonetics™ Cells are guaranteed to perform to our release criteria when cultured with the provided protocol in our recommended media and reagents
- Media systems are offered as BulletKits™ (basal medium and SingleQuots™ Kit) to provide the flexibility to manipulate media components specific to your application, and a longer shelf life prior to use

General Ordering and Shipping Information

Cryopreserved cells and media products are normally shipped Monday – Thursday for next day delivery. Saturday and Monday deliveries are available upon special request.

Proliferating cell orders are processed every other week and must be placed no later than Tuesday at 5:00 pm EST for shipment the following Monday (for delivery on Tuesday). Orders placed after Tuesday will be held until the next production cycle. When you place your order for proliferating cells, please order the appropriate media. Other cell types may be available upon request. Well plate orders require an additional passage and take an extra week.

Cell pellet orders require 7–10 production days. Please plan accordingly.

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Bladder Cells and Media

The bladder serves as a reservoir for water soluble byproducts generated during cell metabolism. Soluble wastes are excreted through the urinary system, which consists of the kidneys, ureters, urinary bladder, and urethra.

Source

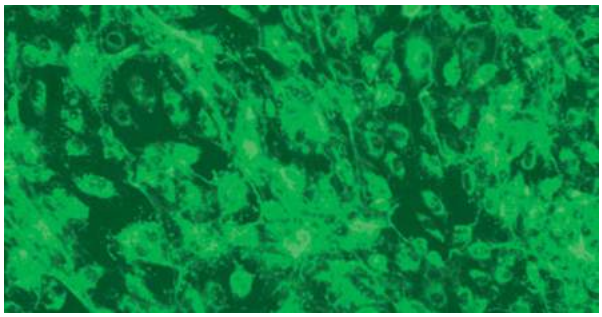
– Human bladder smooth muscle cells and human bladder microvascular endothelial cells both isolated from specific tissues layers surrounding the bladder

Applications

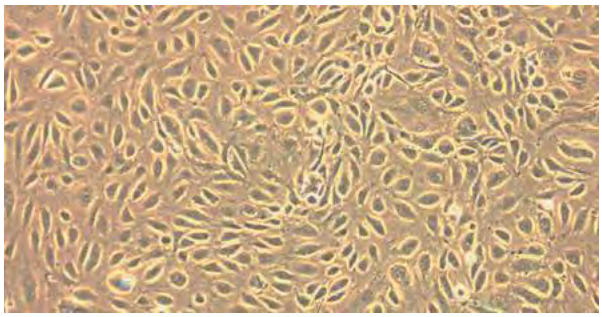
– Overactive bladder
– Cancer
– Urologic disease

Cell Testing and Specifications

– BdSMC stain positive for smooth muscle α-actin and negative for von Willebrand Factor
– HMVEC-Bd stain positive for von Willebrand Factor and LDL and negative for smooth muscle α-actin
– Both cell types are guaranteed through ten population doublings when using Clonetics™ Media and Reagents



HMVEC-Bd culture stained for von Willebrand Factor (green)



HMVEC-Bd at >90% confluency

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
BdSMC	Bladder smooth muscle	SmGM™ 2 BulletKit™	3rd passage	4th passage	3,500 cells/cm^2	6 to 9 days
HMVEC-Bd	Bladder microvascular endothelial	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	6 to 9 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2533	CC-2533	BdSMC – Human Bladder Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-7016	CC-7016	HMVEC-Bd – Human Bladder Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

More ordering information on the next page.

Bladder Cells and Media

Continued

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3182	CC-3182	SmGM™ 2 Smooth Muscle Cell Growth Medium -2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4149	CC-4149	SmGM™ 2 Smooth Muscle Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
	CC-3182 / 6	SmGM™ 2 Smooth Muscle Cell Growth Medium-2 BulletKit™	Six pack, includes basal medium and SingleQuots™ Kit	Kit
CC-3181	CC-3181	SmBM™ Smooth Muscle Cell Basal Medium		500 mL
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
	CC-3202 / 6	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Six pack, includes basal medium and SingleQuots™ Kit	Kit
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3156	CC-3156	EBM™ 2 Endothelial Cell Basal Medium-2		500 mL

See pages 400–409.

Related Products	Page
Cell Culture Reagents	146
Nucleofector™ Kits for Primary Mammalian Smooth Muscle Cells	218
Nucleofector™ Kits for Primary Mammalian Endothelial Cells	207

Cardiac Cells and Media

Cardiac cells are used to study the functions and general pathophysiology of the human cardiovascular system. Some of these cell types are available from normal, Type I and Type II diabetic donors.

Source

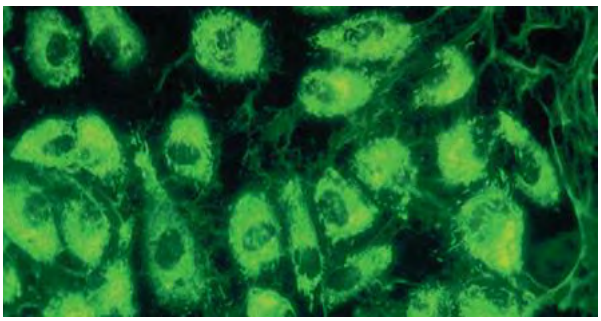
- Human aorta adventitial fibroblasts isolated from tunica external of ascending or descending aorta
- Cardiac fibroblasts isolated from atrial and ventricular cardiac tissue
- Endothelial cells isolated from human aorta, coronary artery and small vessel endothelial cells from ventricle tissue
- Smooth muscle cells isolated from aorta and coronary artery

Applications

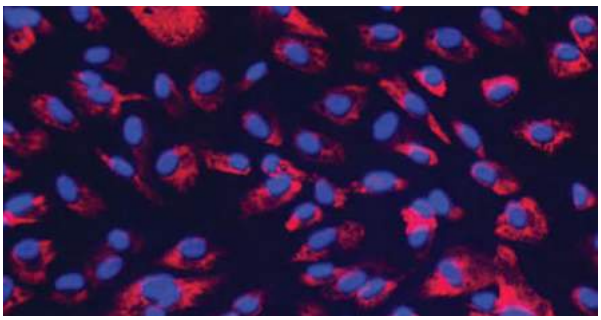
- Arrhythmia
- Cardiomyopathy
- Heart failure
- Preventative cardiology
- Vascular research

Cell Testing and Specifications

- **Endothelial cells** – Positive for acetylated low density lipoprotein LDL uptake, normal healthy endothelial cells take LDL and store in vacuoles; positive for von Willebrand Factor Expression/Factor VIII, positive stain reflects a healthy endothelial cell and von Willebrand Factor synthesis is critical to blood clotting; up to 15 population doublings guaranteed when using Clonetics™ Media and Reagents
- **Fibroblasts** – Cardiac fibroblasts stain positive for collagen I and negative for von Willebrand factor VIII and are guaranteed through five population doublings when using Clonetics™ Media and Reagents, AoAF stain negative for α-actin and are guaranteed through ten population doublings when using Clonetics™ Media and Reagents
- **Smooth muscle cells** – Stain positive for α-actin and negative for von Willebrand Factor after differentiation, and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents



HCAEC culture stained for von Willebrand Factor (green)



Human cardiac fibroblasts (ventricle) at fifth passage stained for collagen (red) and counterstained with DAPI (blue) (20x)

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
HAEC*	Aortic endothelial	EGM™ 2 BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
HCAEC*	Coronary artery	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-C*	Cardiac microvascular	EGM™ 2MV BulletKit™	3rd passage	n/a	5,000 cells/cm^2	5 to 9 days
AoAF	Aortic adventitial fibroblasts	SCGM™ BulletKit™	2nd passage	3rd passage	3,500 cells/cm^2	6 to 9 days
NHCF-A	Atrial cardiac fibroblasts	FGM™-3 BulletKit™	2nd passage	3rd passage	5,000 cells/cm^2	6 to 9 days
NHCF-V	Ventricle cardiac fibroblasts	FGM™-3 BulletKit™	2nd passage	3rd passage	5,000 cells/cm^2	6 to 9 days
AoSMC*	Aortic smooth muscle	SmGM™ 2 BulletKit™	3rd passage	4th or 5th passage	3,500 cells/cm^2	6 to 10 days
CASMC*	Coronary artery	SmGM™ 2 BulletKit™	3rd passage	4th or 5th passage	3,500 cells/cm^2	6 to 10 days
HPAEC*	Pulmonary Artery Endothelial	EGM™ 2 BulletKit™	3rd passage	4th passage	5000 cells/cm^2	5 to 9 days
PASMC*	Pulmonary Artery Smooth Muscle	SmGM™ 2 BulletKit™	3rd passage	4th or 5th passage	3,500 cells/cm^2	6 to 10 days

* Cells also available from Type I and Type II diabetic donors

Cardiac Cells and Media

Continued

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
CC-2535	CC-2535	HAEC – Human Aortic Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2530	CC-2530	HPAEC – Human Pulmonary Artery Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2585	CC-2585	HCAEC – Human Coronary Artery Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-7030	CC-7030	HMVEC-C – Human Cardiac Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2581	CC-2581	HPASMC – Human Pulmonary Artery Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-7014	CC-7014	AoAF – Human Aortic Adventitial Fibroblasts	Cryopreserved, in SCGM™ BulletKit™	≥500,000 cells/vial
CC-2903	CC-2903	NHCF-A – Normal Human Artial Cardiac Fibroblasts	Cryopreserved, in FGM™ 3 BulletKit™	≥500,000 cells/vial
CC-2904	CC-2904	NHCF-V – Normal Human Ventricular Cardiac Fibroblasts	Cryopreserved, in FGM™ 3 BulletKit™	≥500,000 cells/vial
CC-2571	CC-2571	AoSMC – Human Aortic Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2583	CC-2583	CASMC – Human Coronary Artery Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
Diseased Cells				
CC-2919	CC-2919	D-HAEC – Diseased Human Aortic Endothelial – Diabetes Type I	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2920	CC-2920	D-HAEC – Diseased Human Aortic Endothelial – Diabetes Type II	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2921	CC-2921	D-HCAEC – Diseased Human Coronary Artery Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2922	CC-2922	D-HCAEC – Diseased Human Coronary Artery Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2923	CC-2923	D-HPAEC – Diseased Human Pulmonary Artery Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2924	CC-2924	D-HPAEC – Diseased Human Pulmonary Artery Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2927	CC-2927	D-HMVEC-C – Diseased Cardiac Microvascular Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2928	CC-2928	D-HMVEC-C – Diseased Cardiac Microvascular Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2914	CC-2914	D-AoSMC – Diseased Human Aortic Smooth Muscle – Diabetes Type I	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2916	CC-2916	D-AoSMC – Diseased Human Aortic Smooth Muscle – Diabetes Type II	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2917	CC-2917	D-CASMC – Diseased Human Coronary Artery Smooth Muscle – Diabetes Type I	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2918	CC-2918	D-CASMC – Diseased Human Coronary Artery Smooth Muscle – Diabetes Type II	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2915	CC-2915	D-PASMC – Diseased Human Pulmonary Artery Smooth Muscle Cells – Diabetes Type I	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2913	CC-2913	D-PASMC – Diseased Human Pulmonary Artery Smooth Muscle Cells – Diabetes Type II	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial

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Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3156	CC-3156	EBM™ 2 Endothelial Cell Basal Medium-2		500 mL
CC-3162	CC-3162	EGM™ 2 Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4176	CC-4176	EGM™ 2 Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3131	CC-3131	FBM™ Fibroblast Basal Medium		500 mL
CC-4525	CC-4525	FGM™ 3 Cardiac Fibroblast Growth Medium-3 SingleQuots™ Supplements and Growth Factors		Kit
CC-4526	CC-4526	FGM™ 3 Cardiac Fibroblast Growth Medium-3 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3181	CC-3181	SmBM™ Smooth Muscle Cell Basal Medium		500 mL
CC-3182	CC-3182	SmGM™ 2 Smooth Muscle Cell Growth Medium -2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4149	CC-4149	SmGM™ 2 Smooth Muscle Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3204	CC-3204	SCBM™ Stromal Cell Basal Medium		500 mL
CC-3205	CC-3205	SCGM™ Stromal Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4181	CC-4181	SCGM™ Stromal Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

NOTE: Normal cell media is recommended for related disease cell types.

Related Products	Page
CytoSMART™ System	252
RAFT™ 3D Culture System	256
Nucleofector™ Kits for Human Coronary Artery Endothelial Cells (HCAEC)	204
Nucleofector™ Kits for Mammalian Endothelial Cells	207
Nucleofector™ Kits for Mammalian Fibroblasts	213
Nucleofector™ Kits for Human Aortic Smooth Muscle Cells	216

Dermal Cells and Media

We offer a variety of cell types isolated from dermal tissue from normal, Type I and Type II diabetic donors.

Source

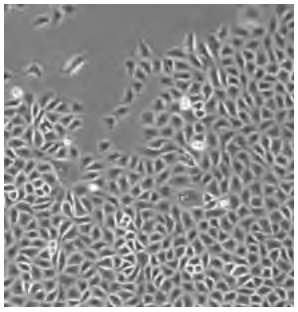
- Small vessel endothelial cells derived from dermal microvascular tissue
- Adult human dermal fibroblasts derived from adult skin tissue and neonatal human dermal fibroblasts derived from neonatal foreskins
- Keratinocytes derived from human neonatal foreskins and adult skin tissue
- Melanocytes derived from human neonatal foreskins and adult skin tissue

Applications

- Epithelial cell model
- Wound healing
- Burn therapy
- Dermatology disorders
- Inflammation
- Drug uptake or drug discovery
- Cell-to-cell junctions
- Cell differentiation
- Viral-induced transformation
- Cancer
- Drug efficacy
- Immunology
- Fibrosis
- Angiogenesis
- Oncology
- Cell signaling
- Cell adhesion
- Pigmentation *(melanogenesis)*

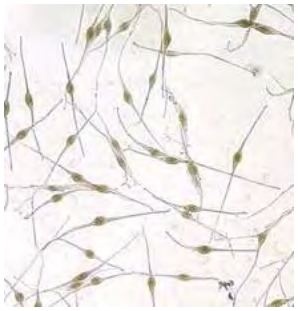
Cell Testing and Specifications

- **Endothelial cells** – Test positive for acetylated low density lipoprotein uptake and positive for von Willebrand Factor Expression/Factor VIII. Up to 15 population doublings are guaranteed for normal cells when using Clonetics™ Media and Reagents
- **Fibroblasts** – NHDF are characterized by morphological observation throughout serial passage and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
- **Keratinocytes** – Are characterized by morphological observation throughout serial passage and are guaranteed through 18–20 population doublings for normal adult and neonatal cells, respectively, when using Clonetics™ Media and Reagents

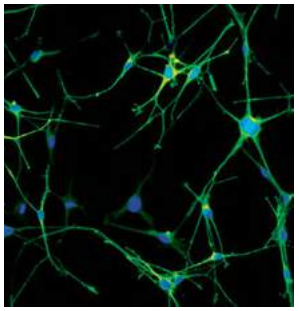


KGM-Gold™ Adult NHEK cells in passage 2

NHDF-excellent, uniform morphology



NHEM stained for L-dopa



NHEM stained for Mel-5 (green) and counter stained for DAPI (blue)

- **Melanocytes** – Are characterized for purity through immunofluorescent labeling of Mel-5 (gp75/TRP-1) with most cultures exceeding 85% Mel-5 labeling, they are also tested for function – 70% of the cells in culture converting L-dopa into dopa-melanin. Their morphology and proliferative capacity is monitored throughout serial passage after recovery from cryopreservation

Ordering information on the next page.

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Dermal Cells and Media

Continued

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
HMVEC-dAd	Adult dermal microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dBlAd	Adult dermal blood microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	4 to 7 days
HMVEC-dBlNeo	Neonatal dermal blood microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	4 to 7 days
HMVEC-dLyAd	Adult dermal lymphatic microvascular	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	4 to 7 days
HMVEC-dNeo	Neonatal dermal microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dLyNeo	Neonatal dermal lymphatic microvascular	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	4 to 7 days
NHDF-Ad	Adult dermal fibroblasts	FGM™ 2 BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	6 to 9 days
NHDF-Neo	Neonatal dermal fibroblasts	FGM™ 2 BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	6 to 9 days
NHEK-Ad	Epidermal keratinocytes, adult	KGM™ Gold BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	5 to 9 days
NHEK-Neo	Epidermal keratinocytes, neonatal	KGM™ Gold BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	5 to 9 days
NHEK-Neo Pooled	Epidermal keratinocytes, neonatal, pooled	KGM™ Gold BulletKit™	1st passage	2nd passage	3,500 cells/cm^3	6 to 9 days
NHEM-Neo	Neonatal normal human epidermal melanocytes	MGM™ 4 BulletKit™	3rd passage	4th passage	10,000 cells/cm^2	9 to 14 days
NHEM-Ad	Adult normal human epidermal melanocytes	MGM™ 4 BulletKit™ + ET-3 Supplement	2nd passage	3rd passage	10,000 cells/cm^2	9 to 14 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
CC-2505	CC-2505	HMVEC-dNeo – Human Dermal Microvascular Endothelial Cells, Neonatal	Cryopreserved, in EGM™ 2MV BulletKit™, single donor	≥500,000 cells/vial
CC-2543	CC-2543	HMVEC-dAd – Human Dermal Microvascular Endothelial Cells – Adult	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2812	CC-2812	HMVEC-dLyNeo – Human Dermal Lymphatic Microvascular Endothelial Cells – Neonatal	Cryopreserved, in EGM™ 2MV BulletKit™, single donor	≥500,000 cells/vial
CC-2811	CC-2811	HMVEC-dBlAd – Human Dermal Blood Microvascular Endothelial Cells – Adult	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2813	CC-2813	HMVEC-dBl-Neo – Human Dermal Blood Microvascular Endothelial Cells – Neonatal	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2509	CC-2509	NHDF-Neo – Normal Human Dermal Fibroblasts – Neonatal	Cryopreserved, in FGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2511	CC-2511	NHDF-Ad – Normal Human Dermal Fibroblasts – Adult	Cryopreserved, in FGM™ 2 BulletKit™	≥500,000 cells/vial
192627	192627	NHEK-Ad™ – Normal Human Epidermal Keratinocytes™ – Adult	Cryopreserved, in KGM™ Gold BulletKit™, single donor	≥500,000 cells/vial
CC-2507	CC-2507	NHEK-Neo - Normal Human Epidermal Keratinocytes - Neonatal	Cryopreserved, in KGM™ BulletKit™, pooled	≥500,000 cells/vial
192906	192906	NHEK-Neo – Normal Human Epidermal Keratinocytes – Neonatal	Cryopreserved, in KGM™-Gold BulletKit™, pooled	≥500,000 cells/vial
192907	192907	NHEK-Neo – Normal Human Epidermal Keratinocytes – Neonatal	Cryopreserved, in KGM™ Gold BulletKit™, single donor	≥500,000 cells/vial
CC-2503	CC-2503	NHEK-Neo – Normal Human Epidermal Keratinocytes – Neonatal	Cryopreserved, in KGM™ Gold BulletKit™	≥500,000 cells/vial
CC-2504	CC-2504	NHEM-Neo – Normal Human Epidermal Melanocytes – Neonatal	Cryopreserved, in MGM™ 4 BulletKit™	≥500,000 cells/vial
CC-2586	CC-2586	NHEM-Ad – Normal Human Epidermal Melanocytes – Adult	Cryopreserved, in MGM™ 4 BulletKit™	≥500,000 cells/vial
Diseased Cells				
CC-2926	CC-2926	D-HEK-Ad – Diseased Human Adult Epidermal Keratinocytes – Diabetes Type II	Cryopreserved, in KGM™-Gold BulletKit™	≥500,000 cells/vial
CC-2929	CC-2929	D-HMVEC-dAD – Diseased Human Dermal Microvascular Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2930	CC-2930	D-HMVEC-dAD – Diseased Human Dermal Microvascular Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Dermal Cells and Media

Continued

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3131	CC-3131	FBM™ Fibroblast Basal Medium		500 mL
CC-3132	CC-3132	FGM™ 2 Fibroblast Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4126	CC-4126	FGM™ 2 Fibroblast Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
192060	192060	KGM™ Gold Keratinocyte Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
192151	192151	KBM™ Gold Keratinocyte Basal Medium		500 mL
192152	192152	KGM™ Gold Keratinocyte Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
195769	195769	KGM™ Gold Keratinocyte Growth Medium BulletKit™	Calcium-free, includes basal medium and SingleQuots™ Kit	Kit
195130	195130	KBM™ Gold Keratinocyte Basal Medium	Without phenol red or calcium	500 mL
CC-4455	CC-4455	TheraPEAK™ KGM™ CD Keratinocyte Growth Medium BulletKit™	Chemically defined, includes basal medium and SingleQuots™ Kit	Kit
CC-4456	CC-4456	KGM™ CD Keratinocyte Growth Medium SingleQuots™ Supplements and Growth Factors	Chemically defined	Kit
CC-3255	CC-3255	KBM™ CD Keratinocyte Basal Medium	Chemically defined	500 mL
CC-3249	CC-3249	MGM™ 4 Melanocyte Growth Medium-4 BulletKit	Includes basal medium and SingleQuots™ Kit	Kit
CC-3250	CC-3250	MBM™ 4 Melanocyte Basal Medium-4		500 mL
CC-4435	CC-4435	MGM™ 4 Melanocyte Growth Medium-4 SingleQuots™ Supplements and Growth Factors		Kit
17-516F	BE17-516F	Phosphate Buffered Saline (1X)	6.7 mM (PO_4) without calcium or magnesium	500 mL
CC-5012	CC-5012	Trypsin/EDTA Solution		100 mL
17-711E	BE17-711E	Versene® (EDTA), 0.02%	0.2 g/L Ethylenediaminetetraacetic acid (0.53 mM) in DPBS, without calcium or magnesium	100 mL
CC-5002	CC-5002	Trypsin Neutralizing Solution		100 mL
CC-4510	CC-4510	Endothelin-3 (ET-3) Growth Supplement		130 µg
10-547F	BE10-547F	Hank's Buffered Saline Solution	Without phenol red, calcium or magnesium	500 mL

See pages 400–409.

Endothelial cells must be cultured in their isolation medium for best results.

NOTE: Normal cell media is recommended for related disease cell types.

Related Products	Page
CytoSMART™ System	252
RAFT™ 3D Culture System	256
Nucleofector™ Kits for Primary Dermal Cells	202
Nucleofector™ Kits for Primary Mammalian Endothelial Cells	207
Nucleofector™ Kits for Human Dermal Fibroblasts	211

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Large Vessel Endothelial Cells and Media

Endothelial cells line the inside surface of blood vessels, heart, lymphatic vessels, body cavities and other organs of normal human tissue.
We offer many of these cell types from normal, Type I and Type II diabetic donors.

Source

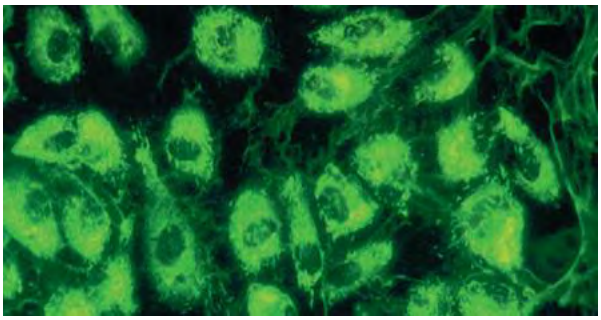
– Large vessel endothelial cells are isolated from the human aorta, umbilical artery and vein, and coronary, iliac and pulmonary arteries

Applications

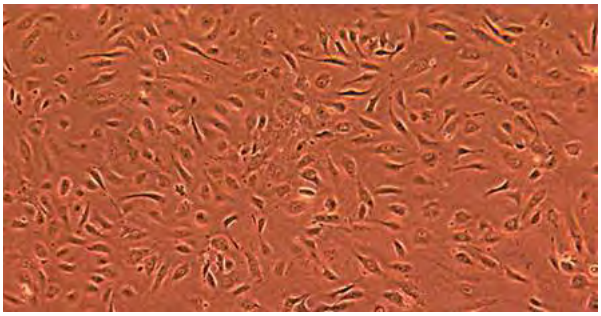
– Atherosclerosis
– Arteriosclerosis
– Drug uptake or drug discovery
– Wound healing
– Angiogenesis
– Inflammation
– Oncology

Cell Testing and Specifications

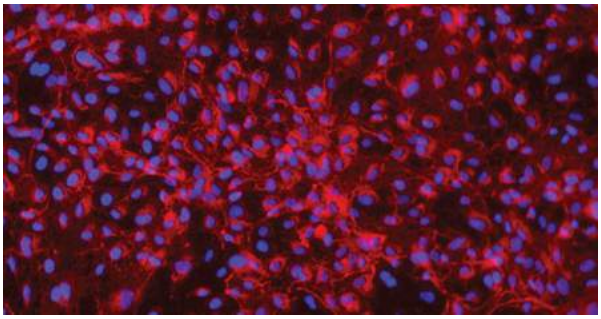
– **Endothelial cells** – Test positive for acetylated low density lipoprotein uptake; positive for von Willebrand Factor Expression/Factor VIII. HUVEC test ≥90% double positive for CD31/CD105 markers by flow cytometry. Up to 15 population doublings are guaranteed with normal cells when using Clonetics™ Media and Reagents. Up to 5 population doublings are guaranteed for HUVEC-XL when using Clonetics™ Media and Reagents.
– **Prescreened HUVECs** – Isolated in EGM™ 2 medium, pooled from 3 to 5 donors, and tested for angiogenesis/endothelial health related markers: Axl, eNOS, Tie-2, and VEGFr2.



HCAEC culture stained for von Willebrand Factor



HAEC at >90% confluency



HUVEC stained for von Willebrand Factor and counterstained with DAPI

New! Clonetics™ HUVECs cultured without additive VEGF and EGM™ Plus Growth Media Bulletkit™

Our new EGM™ Plus Growth Media BulletKit™ is now available to support HUVECs without additive VEGF. HUVECs cultured in EGM™ Plus are an improved version of HUVECs cultured in EGM™ Media. HUVECs cultured in EGM™ Plus offer faster proliferation rates while maintaining the same high quality characterization as HUVECs in EGM™ Media.

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
HAEC	Aortic endothelial	EGM™ 2 BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
HCAEC	Coronary artery	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
HIAEC	Iliac artery endothelial	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
HPAEC	Pulmonary artery	EGM™ 2 BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
HUVEC	Umbilical vein	NEW EGM™ Plus BulletKit™	1st passage	2nd passage	2,500 cells/cm^2	5 to 7 days
HUVEC	Umbilical vein	EGM™ 2 or EGM™ BulletKit™	1st passage	2nd passage	2,500 cells/cm^2	5 to 9 days
HUVEC-XL	Umbilical vein	EGM™ 2 BulletKit™	3rd passage	n/a	2,500 cells/cm^2	5 to 9 days

Large Vessel Endothelial Cells and Media

Continued

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
CC-2535	CC-2535	HAEC – Human Aortic Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2585	CC-2585	HCAEC – Human Coronary Artery Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2545	CC-2545	HIAEC – Human Iliac Artery Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2530	CC-2530	HPAEC – Human Pulmonary Artery Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2935	CC-2935	NEW HUVEC – Umbilical Vein Endothelial Cells without VEGF, single donor	Cryopreserved, in EGM™ Plus BulletKit™	≥500,000 cells/vial
C2517A	C2517A	HUVEC – Human Umbilical Vein Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™, single donor	≥500,000 cells/vial
C2517AS	C2517AS	HUVEC – Human Umbilical Vein Endothelial Cells	Pre-screened, in EGM™ 2 BulletKit™, single donor	≥500,000 cells/vial
C2519A	C2519A	HUVEC – Human Umbilical Vein Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™, pooled	≥500,000 cells/vial
C2519AS	C2519AS	HUVEC – Human Umbilical Vein Endothelial Cells	Pre-screened, in EGM™ 2 BulletKit™, pooled	≥500,000 cells/vial
CC-2517	CC-2517	HUVEC – Human Umbilical Vein Endothelial Cells	Cryopreserved, in EGM™ BulletKit™, single donor	≥500,000 cells/vial
CC-2519	CC-2519	HUVEC – Human Umbilical Vein Endothelial Cells	Cryopreserved, in EGM™ BulletKit™, pooled	≥500,000 cells/vial
191027	191027	HUVEC-XL – Human Umbilical Vein Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™, expanded, pooled	≥10 million cells/vial
Diseased Cells				
CC-2919	CC-2919	D-HAEC – Diseased Human Aortic Endothelial – Diabetes Type I	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2920	CC-2920	D-HAEC – Diseased Human Aortic Endothelial – Diabetes Type II	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2921	CC-2921	D-HCAEC – Diseased Human Coronary Artery Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2922	CC-2922	D-HCAEC – Diseased Human Coronary Artery Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2923	CC-2923	D-HPAEC – Diseased Human Pulmonary Artery Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2924	CC-2924	D-HPAEC – Diseased Human Pulmonary Artery Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

NOTE: Normal cell media is recommended for related disease cell types.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3162	CC-3162	EGM™ 2 Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3156	CC-3156	EBM™ 2 Endothelial Cell Basal Medium-2		500 mL
CC-4176	CC-4176	EGM™ 2 Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3024	CC-3024	EGM™ Complete Endothelial Cell Growth Medium	With 2% FBS	500 mL
CC-3121	CC-3121	EBM™ Endothelial Cell Basal Medium		500 mL
CC-3129	CC-3129	EBM™ PRF Endothelial Cell Basal Medium	Phenol red-free	500 mL
CC-4133	CC-4133	EGM™ Endothelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5035	CC-5035	NEW EGM™ Plus Endothelial cells Growth Media BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-5036	CC-5036	NEW EBM™ Plus Endothelial Cell Basal Medium		475 ml
CC-4542	CC-4542	NEW EGM™ Plus SingleQuot™ Kits and Growth Supplements		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

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Microvascular Endothelial Cells and Media

Endothelial cells line the inside surface of blood vessels, heart, lymphatic vessels, body cavities and other organs, of normal human tissue.
We offer a variety of cell types isolated from microvascular tissue from normal, Type I and Type II diabetic donors.

Source

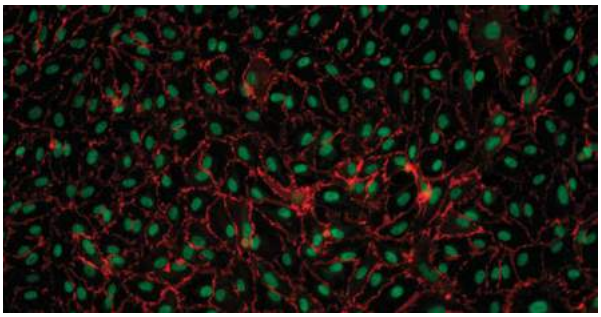
– Small vessel endothelial cells are isolated from dermal, lung, cardiac and uterine microvascular tissue

Applications

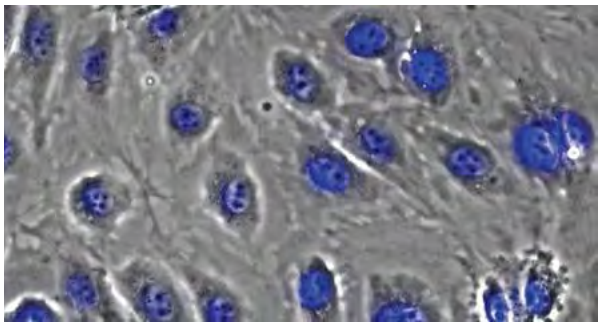
– Atherosclerosis
– Angiogenesis
– Arteriosclerosis
– Drug uptake or drug discovery
– Cell-to-cell junctions
– Inflammation
– Wound healing
– Oncology

Cell Testing and Specifications

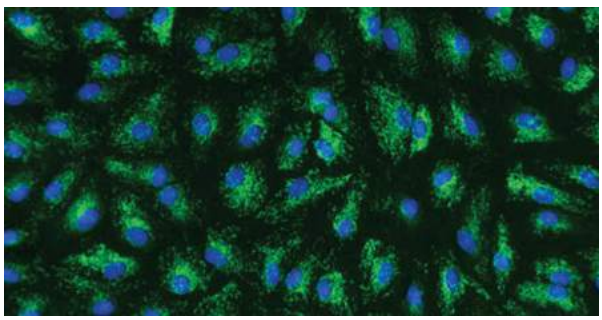
– **Endothelial cells** – Test positive for acetylated low density lipoprotein uptake; positive for von Willebrand Factor Expression/Factor VIII; and PECAM-positive for lung microvascular cells. PECAM-positive stain confirms presence of adherent junctions which control passage of molecules across tissues. Up to 15 population doublings are guaranteed with normal cells when using Clonetics™ Media and Reagents



HMVEC-dLyAd stained CD31(red)/Prox-1(green)



HMVEC-dAd Hoechst stain



HMVEC-LLy von Willebrand Factor(green) and DAPI stain(blue)

Microvascular Endothelial Cells and Media

Continued

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
HMVEC-C	Cardiac microvascular	EGM™ 2MV BulletKit™	3rd passage	n/a	5,000 cells/cm^2	5 to 9 days
HMVEC-L	Lung microvascular	EGM™ 2MV BulletKit™	3rd or 4th passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dAd	Adult dermal microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dBlAd	Adult dermal blood microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dBlNeo	Neonatal dermal blood microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dLyAd	Adult dermal lymphatic microvascular	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dNeo	Neonatal dermal microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HMVEC-dLyNeo	Neonatal dermal lymphatic microvascular	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	5 to 9 days
UtMVEC-Myo	Uterine microvascular	EGM™ 2MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
CC-7016	CC-7016	HMVEC-Bd – Human Bladder Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-7030	CC-7030	HMVEC-C – Human Cardiac Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2543	CC-2543	HMVEC-dAd – Human Dermal Microvascular Endothelial Cells – Adult	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2813	CC-2813	HMVEC-dBl-Neo – Human Dermal Blood Microvascular Endothelial Cells – Neonatal	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2811	CC-2811	HMVEC-dBlAd – Human Dermal Blood Microvascular Endothelial Cells – Adult	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2810	CC-2810	HMVEC-dLyAd – Human Dermal Lymphatic Microvascular Endothelial Cells – Adult	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2812	CC-2812	HMVEC-dLyNeo – Human Dermal Lymphatic Microvascular Endothelial Cells – Neonatal	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2505	CC-2505	HMVEC-dNeo – Human Dermal Microvascular Endothelial Cells, neonatal	Cryopreserved, in EGM™ 2MV BulletKit™, single donor	≥500,000 cells/vial
CC-2516	CC-2516	HMVEC-dNeo – Human Dermal Microvascular Endothelial Cells, neonatal	Cryopreserved, in EGM™ 2MV BulletKit™, pooled	≥500,000 cells/vial
CC-2527	CC-2527	HMVEC-L – Human Lung Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2564	CC-2564	UtMVEC-Myo – Human Uterine Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
Diseased Cells				
CC-2927	CC-2927	D-HMVEC-C – Diseased Cardiac Microvascular Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2928	CC-2928	D-HMVEC-C – Diseased Cardiac Microvascular Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2929	CC-2929	D-HMVEC-dAD – Diseased Human Dermal Microvascular Endothelial Cells – Diabetes Type I	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2930	CC-2930	D-HMVEC-dAD – Diseased Human Dermal Microvascular Endothelial Cells – Diabetes Type II	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

More ordering information on the next page.

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Microvascular Endothelial Cells and Media

Continued

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3156	CC-3156	EBM™ 2 Endothelial Cell Basal Medium-2		500 mL
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3125	CC-3125	EGM™ MV Microvascular Endothelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3121	CC-3121	EBM™ Endothelial Cell Basal Medium		500 mL
CC-4143	CC-4143	EGM™ MV Microvascular Endothelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–409.

Endothelial cells must be cultured in their isolation medium for best results.

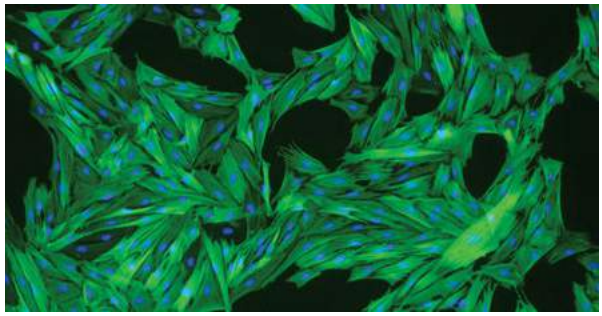
NOTE: Normal cell media is recommended for related disease cell types.

Related Products	Page
CytoSMART™ System	252
RAFT™ 3D Culture System	256
Nucleofector™ Kits for Primary Mammalian Endothelial Cells	207

Gastrointestinal Cells and Media

The gastrointestinal tract breaks down food into nutrients and smaller molecules, which are either absorbed into the body to provide energy or expelled as a waste. Digestion occurs mainly in the stomach and small intestine. Small molecules are absorbed across the epithelium of the small intestine and later enter the bloodstream to carry nutrients to other parts of the body. Intestinal myofibroblasts reside subjacent to the basal membrane in the intestines and mediate molecular flow between the epithelium and cells in the lamina propria.

Our cryopreserved InEpC are truly primary cells representing both villi (enterocytes, goblet, and enteroendocrine cells) and crypts structures.



Human intestinal myofibroblasts at second passage stained for α-smooth muscle actin (green) and counterstained with DAPI (blue)

Source

– Human small intestine, specifically the jejunum

Applications

– Gastrointestinal disease or disorder
– Oncology
– Cell physiology
– Drug discovery
– Toxicology and cytotoxicity

Cell Testing and Specifications

– **Gastrointestinal myofibroblasts** – Test ≥90% positive for α-smooth muscle actin and ≤10% positive for the expression of desmin. Human myofibroblasts are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents.
– **Intestinal epithelial cells** – test ≥90% positive for cytokeratins 8/18. These cells cannot be subcultured. In combination with human intestinal myofibroblasts (InMyoFib), InEpC are able to form very tight cell monolayer, representing a unique *in vitro* system to model human intestinal homeostasis.

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
InMyoFib	Intestinal Myofibroblasts	SmGM™ 2 BulletKit™	2nd passage	3rd passage	2,500 cells/cm^2	5 to 7 days
InEpi	Intestinal Epithelial Cells	SmGM™ 2 BulletKit™	Immediate Passage	-	150,000 viable cells/cm^2	n/a

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2902	CC-2902	InMyoFib – Human Intestinal Myofibroblasts	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2931	CC-2931	InEpC – Human Intestinal Epithelial Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥800,000 viable cells/vial
CC-4540	CC-4540	Human Intestinal Epithelial and Myofibroblast Cell Combo	Includes one amp each CC-2902 and CC-2931	

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3182	CC-3182	SmGM™ 2 Smooth Muscle Cell Growth Medium -2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit

See pages 400–409.

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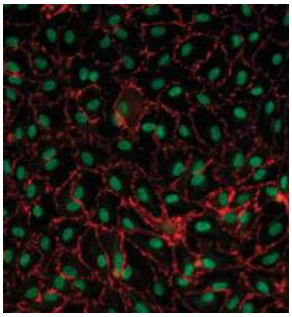
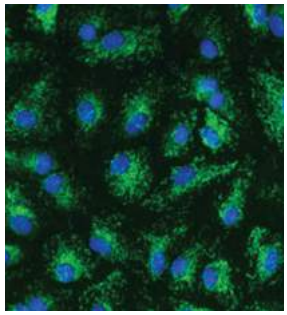
Lymphatic Cells and Media

Endothelial cells are found in the membrane or monolayer lining of cells taken from lymphatic vessels of heart, lymphatic tissue, surface spinal cord and brain, or anterior eye chamber of normal human tissue.



HMVEC-dLyAd stained CD31 (red)/ Prox-1 (green)



HMVEC-LLy von Willebrand Factor (green) and DAPI stain (blue)

Source

– Lymphatic endothelial cells are isolated from dermal or lung microvascular tissue

Applications

– Inflammation
– Wound healing
– Drug uptake or drug discovery
– Oncology
– Cell-to-cell junctions

Cell Testing and Specifications

– **Endothelial cells** – Test positive for acetylated low density lipoprotein uptake; positive for von Willebrand Factor Expression/Factor VIII; PECAM-positive for lung microvascular cells which stains for adherent junctions that endothelial cells use to control passage of molecules across the tissue. Up to 15 population doublings guaranteed when using Clonetics™ Media and Reagents

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
HMVEC-dLyAd	Adult dermal lymphatic microvascular	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	4 to 7 days
HMVEC-dLyNeo	Neonatal dermal lymphatic microvascular	EGM™ 2MV BulletKit™	3rd passage	4th passage	5,000 cells/cm^2	4 to 7 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2812	CC-2812	HMVEC-dLyNeo – Human Dermal Lymphatic Microvascular Endothelial Cells – Neonatal	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2810	CC-2810	HMVEC-dLyAd – Human Dermal Lymphatic Microvascular Endothelial Cells – Adult	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3156	CC-3156	EBM™ 2 Endothelial Cell Basal Medium-2		500 mL
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit

See pages 400–409.

Related Products	Page
CytoSMART™ System	252
Nucleofector™ Kits for Primary Mammalian Endothelial Cells	207

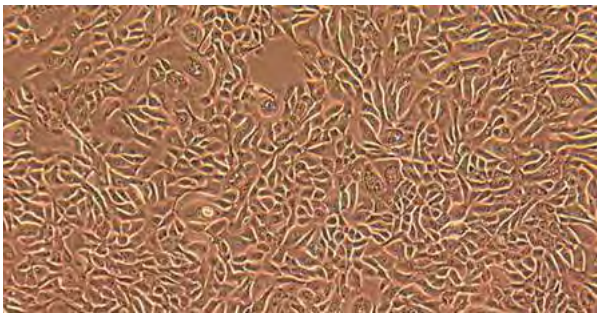
Mammary Epithelial Cells and Media

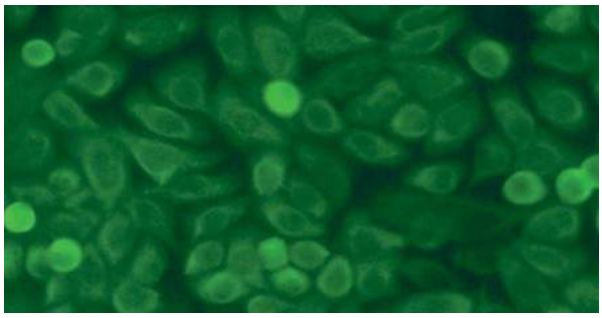
Mammary epithelial cells are isolated from glandular tissue in adult human breast tissue. Cells undergo changes in morphology and function throughout adulthood especially during pregnancy and lactation.

Source
– Human adult breast tissue

Applications
– Breast cancer
– Cellular function and differentiation
– Physiology
– Toxicology
– Hormone regulation and response

Cell Testing and Specifications
– **Human mammary epithelial cells** – Test positive for cytokeratins 14 and 18, and negative for cytokeratin 19 and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents


HMEC 95% confluent


HMEC stained for cytokeratin 18 (green)

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
HMEC	Mammary Epithelial	MEGM™ BulletKit™	6th or 7th passage	7th or 8th passage	2,500 cell/cm^2	6 to 9 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2551	CC-2551	HMEC – Human Mammary Epithelial Cells	Cryopreserved, in MEGM™ BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3150	CC-3150	MEGM™ Mammary Epithelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3151	CC-3151	MEBM™ Mammary Epithelial Cell Basal Medium		500 mL
CC-3152	CC-3152	MEBM™ Mammary Epithelial Cell Basal Medium	Sodium bicarbonate-free	500 mL
CC-3153	CC-3153	MEBM™ Mammary Epithelial Cell Basal Medium	Phenol red-free	500 mL
CC-3051	CC-3051	MEGM™ Complete Mammary Epithelial Cell Growth Medium		500 mL
CC-4136	CC-4136	MEGM™ Mammary Epithelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–409.

Related Products	Page
CytoSMART™ System	252
Nucleofector™ Kits for Human Mammary Epithelial Cells	209

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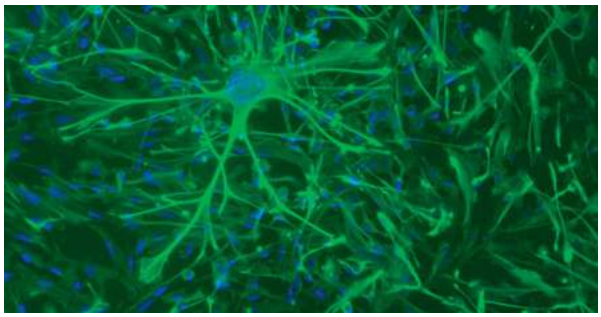
Neural Cells and Media

Clonetics™ Neural Cells are used to study the function of the central nervous system and how neural cells interact in normal tissue. Astrocytes are glial cells found in the brain and spinal cord that play a critical role in maintenance, support and repair of nervous tissue.



NHA stained positive for GFAP(green) and counterstained DAPI (blue)

Source

– Human brain cortex

Applications

– Neurogenesis research
– Pharmacology
– Cell physiology
– Parkinson's disease
– Injury
– Astrocyte-mediated neurotoxicity
– Alzheimer's disease

Cell Testing and Specifications

– **Normal human astrocytes** – Test positive for GFAP and are guaranteed through 10 population doublings when using Clonetics™ Media and Reagents

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
NHA	Astrocytes	AGM™ BulletKit™	1st passage	2nd passage	5,000 cells/cm^2	6 to 8 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2565	CC-2565	NHA – Human Astrocytes	Cryopreserved, in AGM™ BulletKit™	≥1 million cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3186	CC-3186	AGM™ Astrocyte Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3187	CC-3187	ABM™ Astrocyte Basal Medium		200 mL
CC-4123	CC-4123	AGM™ Astrocyte Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–418.

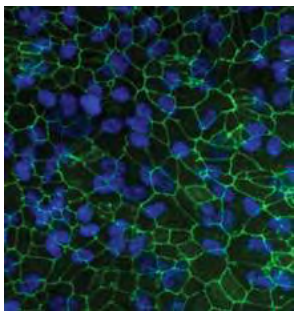
Related Products	Page
RAFT™ 3D Culture System	256
Rat and Mouse Neural Cells	96
Adherent Nucleofection	172, 184
Nucleofector™ Kits for Mammalian Glial Cells	223

Ocular Cells and Media

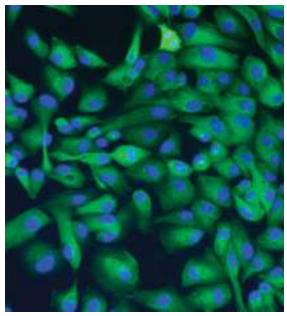
Primary RPE cells are hexagonal cells that are densely packed with pigment granules. They play a critical role in visual function and photoreceptor viability.

Source
– Human eye tissue

Applications
– Proliferative retinopathy
– Age related macular degeneration
– Retinitis pigmentosa
– Stargardt's disease
– Blood-retinal barrier research
– Toxicology and cytotoxicity
– Diabetic retinopathy
– Blindness



RPE cells at passage 3 stained for tight junction marker ZO-1 (green) and counterstained DAPI (blue)



RPE cells at stained for pan Cytokeratin (green), counter-stained with DAPI(blue)

Cell Testing and Specifications
– **RPE cells** – Test ≥90% positive for pancytokeratin marker, ≤10% positive for fibroblast contamination, ≥90% for tight conjunction marker and ≤1% positive for endothelial marker CD31. RPE cells are guaranteed through 5 population doublings when using Clonetics™ Media and Reagents

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
h-RPE	Retinal pigment epithelial cells	RtEGM™ BulletKit™	2nd passage	3rd passage	10,000 cells/cm^2	5 to 7 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
194987	194987	H-RPE – Human Retinal Pigment Epithelial Cells	Cryopreserved, in RtEGM™ BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
195409	195409	RtEGM™ Retinal Pigment Epithelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
195406	195406	RtEBM™ Retinal Epithelial Cell Basal Medium		500 mL
195407	195407	RtEGM™ Retinal Epithelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit

See pages 400–406.

Related Products	Page
CytoSMART™ System	252
Rat Retinal Cells	97

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Pancreatic Islets

Pancreatic islets are hormone-producing regions in pancreas. These islets consist of beta cells which produce insulin in the body. Pancreatic islets are being utilized in diabetes research as these islets restore beta-cell function yielding better regulation of insulin levels.

Source

– Islets are isolated from endocrine regions of the pancreas

Applications

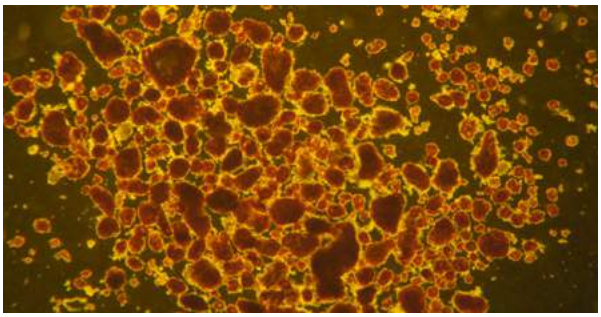
– Islet Grafting Survival
– Immunosuppression
– Insulin Production
– Cell Metabolism
– Diabetes (Type I and Type II); Hypoglycemia

Cell Testing and Specifications

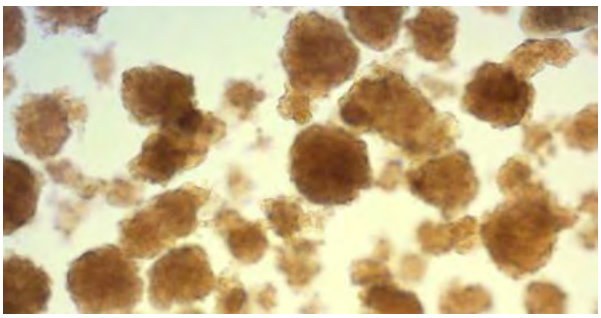
– Islets are tested for IEQ count, sterility, purity and viability prior to shipment. Each batch tests negative for HIV-1, Hep B & C.

Pancreatic Islets are custom ordered through our Cells on Demand™ Service.

Please visit www.lonza.com/islets or contact us at cellsondemand@lonza.com



Pancreatic islets stained with dithizone (DTZ)



Pancreatic islets

Prostate Cells and Media

Prostate cells provide a glandular function in the body by generating fluid which serves several functions in reproduction.

Source

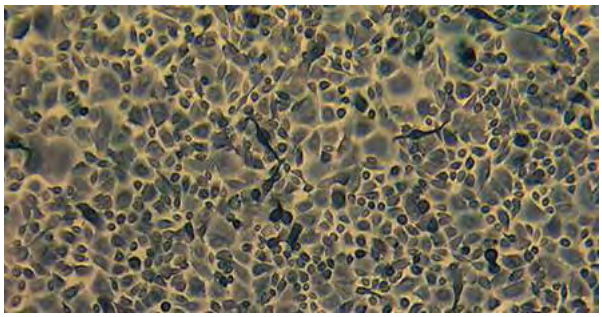
– Prostate epithelial, stromal and smooth muscle tissue depending on cell type

Applications

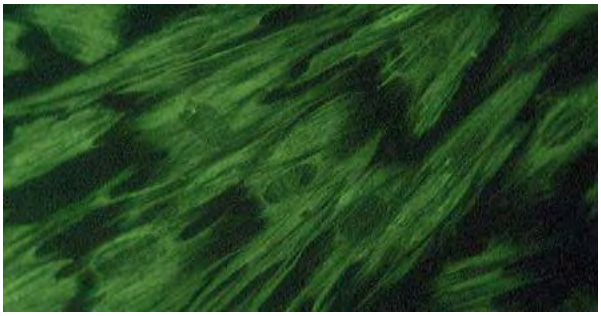
– Physiology
– Drug discovery
– Cancer research
– Procreation research

Cell Testing and Specifications

– **Prostate epithelial cells** – Test positive for cytokeratin (clone 8.13), prostate stromal cells test positive for vimentin and negative for pan cytokeratin. Both epithelial and stromal cell types are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
– **Prostate smooth muscle cells** – Stain positive for α-actin and are guaranteed through 10 population doublings when using Clonetics™ Media and Reagents



PrEC – peroxidase stain for cytokeratin, clone 8.13



PrSC stained for vimentin (green)

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
PrEC	Prostate epithelial	PrEGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm^2	6 to 9 days
PrSC	Prostate stromal	SCGM™ BulletKit™	3rd or 4th passage	4th or 5th passage	3,500 cells/cm^2	6 to 9 days
PrSMC	Prostate smooth muscle	SmGM™ 2 BulletKit™	2nd or 3rd passage	3rd or 4th passage	3,500 cells/cm^2	6 to 9 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2508	CC-2508	PrSC – Human Prostate Stromal Cells	Cryopreserved, in SCGM™ BulletKit™	≥500,000 cells/vial
CC-2555	CC-2555	PrEC – Human Prostate Epithelial Cells	Cryopreserved, in PrEGM™ BulletKit™	≥500,000 cells/vial
CC-2587	CC-2587	PrSMC – Human Prostate Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

More ordering information on the next page.

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Prostate Cells and Media

Continued

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3166	CC-3166	PrEGM™ Prostate Epithelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3165	CC-3165	PrEBM™ Prostate Epithelial Cell Basal Medium		500 mL
CC-4177	CC-4177	PrEGM™ Prostate Epithelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3205	CC-3205	SCGM™ Stromal Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3204	CC-3204	SCBM™ Stromal Cell Basal Medium		500 mL
CC-4181	CC-4181	SCGM™ Stromal Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3181	CC-3181	SmBM™ Smooth Muscle Cell Basal Medium		500 mL
CC-4149	CC-4149	SmGM™ 2 Smooth Muscle Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

PrEGM™ media, see page 406, SCGM™ media, see page 409.

Related Products	Page
CytoSMART™ System	252
Cell Culture Reagents	146
Sertoli Cells	83
Nucleofector™ Kits for Primary Mammalian Epithelial Cells	210
Nucleofector™ Kits for Primary Mammalian Smooth Muscle Cells	218

Pulmonary Cells and Media

Pulmonary cells are found in the lungs and can be used to study respiration including cilia movement, mucus production, gas exchange, air movement, and pulmonary vascular physiology.
We offer these airway cell types from normal, asthma, and COPD diagnosed donors. We have cells from Cystic Fibrosis donors when tissue becomes available.

Source

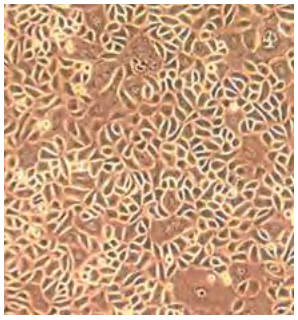
- Human small airway epithelial cells isolated from the distal portion of the lung in the 1 mm bronchiole area
- Human bronchial/tracheal epithelial cells isolated from the epithelial cells that line the airway above the bifurcation of the lungs
- Small vessel endothelial cells are isolated from lung microvascular tissue
- Human lung fibroblasts are isolated from adult lung tissue
- Human bronchial smooth muscle cells are isolated from the major bronchia
- Diseased cell types taken from donors that were diagnosed with either asthma or COPD. Certain characteristics of diseased samples may vary; please contact Scientific Support for further donor information

Applications

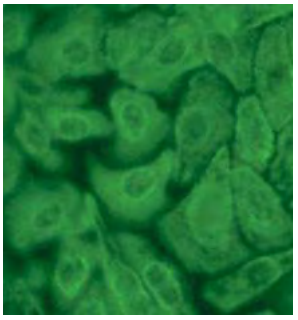
- Cystic fibrosis
- Respiratory disease
- Air /Liquid interface
- COPD
- Respiratory distress syndrome
- Oncology
- Inhalation technology
- Asthma
- Basic research
- Drug uptake studies

Cell Testing and Specifications

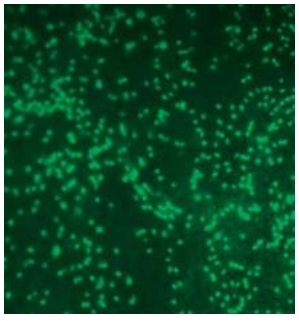
- **Human bronchial/tracheal epithelial cells and small airway epithelial cells** – Characterized by morphological observation throughout serial passage and SAEC stain positive for cytokeratin 19, both are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
- **Human lung fibroblasts** – Test negative for von Willebrand Factor Expression/Factor VIII, cytokeratins 18 and 19, and smooth muscle α-actin and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
- **Smooth muscle cells** – Stain positive for α-actin and negative for von Willebrand Factor Expression/Factor VIII after differentiation and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents



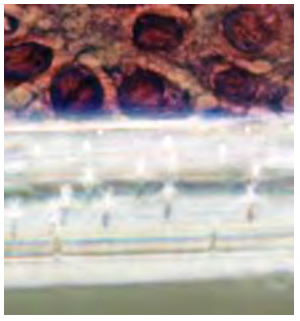
NHBE – Excellent packed cuboidal morphology



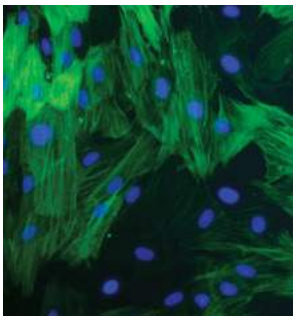
SAEC – Stained for Cytokeratin 19 (green)



NHBE – 25 days post air lift grown in B-ALI™ BulletKit™ stained for cilia with β-tubulin (green)



NHBE – Cross section on membrane, day 26 post air lift grown in B-ALI™ BulletKit™



BSMC – Stained for α smooth muscle actin (green), counter-stained with DAPI (blue)

- **Endothelial cells** – Test positive for acetylated low density lipoprotein uptake; von Willebrand Factor Expression/Factor VIII; and PECAM-positive for lung microvascular cells. PECAM-positive stain confirms presence of adherent junctions which control passage of molecules across tissues. Up to 15 population doublings are guaranteed when using Clonetics™ Media and Reagents; individual cell types may vary

Ordering Information on the next page.

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Pulmonary Cells and Media

Continued

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
SAEC	Small airway epithelial	SAGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm^2	5 to 9 days
D-SAEC-As	Diseased Small Airway Epithelial Cells – Asthma	SAGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm^2	5 to 9 days
D-SAEC-CF	Diseased Small Airway Epithelial Cells – Cystic Fibrosis	SAGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm^2	5 to 9 days
D-SAEC-COPD	Diseased Small Airway Epithelial Cells – COPD	SAGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm2	5 to 9 days
NHBE with RA	Bronchial/Tracheal epithelial	BEGM™ BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	6 to 9 days
NHBE without RA	Bronchial/Tracheal epithelial	BEGM™ BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	6 to 9 days
DHBE-As	Diseased Bronchial/Tracheal epithelial – Asthma	BEGM™ BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	6 to 9 days
DHBE-COPD	Diseased Bronchial/Tracheal epithelial – COPD	BEGM™ BulletKit™	1st passage	2nd passage	3,500 cells/cm^2	6 to 9 days
DHBE-CF	Diseased Bronchial/Tracheal epithelial - Cystic Fibrosis	BEGM™ BulletKit™	2nd passage	3rd passage	3,500 cells/cm^2	6 to 9 days
NHLF	Lung fibroblasts	FGM™ 2 BulletKit™	2nd passage	3rd passage	2,500 cells/cm^2	6 to 9 days
DHLF-As	Diseased Lung fibroblasts – Asthma	FGM™ 2 BulletKit™	2nd passage	3rd passage	2,500 cells/cm^2	6 to 9 days
DHLF-COPD	Diseased Lung fibroblasts – COPD	FGM™ 2 BulletKit™	2nd passage	3rd passage	2,500 cells/cm^2	6 to 9 days
DHLF-CF	Diseased Lung fibroblasts – Cystic Fibrosis	FGM™ 2 BulletKit™	2nd passage	3rd passage	2,500 cells/cm^2	6 to 9 days
HMVEC-L	Lung microvascular	EGM™ 2MV BulletKit™	3rd or 4th passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
HPAEC	Pulmonary artery	EGM™ 2 BulletKit™	3rd passage	4th passage	2,500–5,000 cells/cm^2	5 to 9 days
BSMC	Bronchial SMC	SmGM™ 2 BulletKit™	2nd passage	3rd or 4th passage	3,500 cells/cm^2	6 to 10 days
DBSMC-As	Diseased Bronchial SMC – Asthma	SmGM™ 2 BulletKit™	2nd passage	3rd or 4th passage	3,500 cells/cm^2	6 to 10 days
DBSMC-COPD	Diseased Bronchial SMC – COPD	SmGM™ 2 BulletKit™	2nd passage	3rd or 4th passage	3,500 cells/cm^2	6 to 10 days
DBSMC-CF	Diseased Bronchial SMC – Cystic Fibrosis	SmGM™ 2 BulletKit™	3rd passage	4th passage	3,500 cells/cm^2	6 to 10 days
PASMC	Pulmonary Artery Smooth Muscle	SmGM™ 2 BulletKit™	3rd passage	4th or 5th passage	3,500 cells/cm^2	6 to 10 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
CC-2547	**CC-2547**	SAEC – Human Small Airway Epithelial Cells	Cryopreserved, in SAGM™ BulletKit™	≥500,000 cells/vial
CC-2540	**CC-2540**	NHBE – Human Bronchial/Tracheal Epithelial Cells	Cryopreserved, in BEGM™ BulletKit™, isolated and cultured with retinoic acid	≥500,000 cells/vial
CC-2540S	**CC-2540S**	NHBE – Normal Human Bronchial/Tracheal Epithelial Cells for B-ALI™ Bronchial Air Liquid Interface	Cryopreserved, in BEGM™ BulletKit™, isolated and cultured with retinoic acid	≥500,000 cells/vial
CC-2541	**CC-2541**	NHBE – Human Bronchial/Tracheal Epithelial Cells	Cryopreserved, in BEGM™ BulletKit™, isolated and cultured with retinoic acid	≥500,000 cells/vial
CC-2512	**CC-2512**	NHLF – Normal Human Lung Fibroblasts	Cryopreserved, in FGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2527	**CC-2527**	HMVEC-L – Human Lung Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2530	**CC-2530**	HPAEC – Human Pulmonary Artery Endothelial Cells	Cryopreserved, in EGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2581	**CC-2581**	HPASMC – Human Pulmonary Artery Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2576	CC-2576	BSMC – Human Bronchial Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Pulmonary Cells and Media

Continued

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Diseased Cells				
CC-2932	CC-2932	D-SAEC-As – Diseased Small Airway Epithelial Cells – Asthma	Cryopreserved, in SAGM™ BulletKit™	≥500,000 cells/vial
CC-2933	CC-2933	D-SAEC-CF – Diseased Small Airway Epithelial Cells – Cystic Fibrosis	Cryopreserved, in SAGM™ BulletKit™	≥500,000 cells/vial
CC-2934	CC-2934	D-SAEC-COPD – Diseased Small Airway Epithelial Cells – COPD	Cryopreserved, in SAGM™ BulletKit™	≥500,000 cells/vial
194850	194850	D-BSMC-As – Diseased Bronchial Smooth Muscle Cells – Asthma	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
195274	195274	D-BSMC-COPD – Diseased Bronchial Smooth Muscle Cells – COPD	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
196980	196980	D-HBSMC-CF – Diseased Human Bronchial Smooth Muscle Cells – Cystic Fibrosis	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
196979	196979	D-HBE-CF – Diseased Human Bronchial/Tracheal Epithelial Cells – Cystic Fibrosis	Cryopreserved, in BEGM™ BulletKit™	≥500,000 cells/vial
194911	194911	D-HBE-As – Diseased Human Bronchial/Tracheal Epithelial Cells – Asthma	Cryopreserved, in BEGM™ BulletKit™	≥500,000 cells/vial
194911S	194911S	D-HBE-As – Diseased Human Bronchial/Tracheal Epithelial Cells – Asthma for B-ALI™ Bronchial Air Liquid Interface	Cryopreserved, in BEGM™ BulletKit™	≥500,000 cells/vial
195275	195275	D-HBE-COPD – Diseased Human Bronchial/Tracheal Epithelial Cells – COPD	Cryopreserved, in BEGM™ BulletKit™	≥500,000 cells/vial
195275S	195275S	D-HBE-COPD – Diseased Human Bronchial/Tracheal Epithelial Cells – COPD for B-ALI™ Bronchial Air Liquid Interface	Cryopreserved, in BEGM™ BulletKit™	≥500,000 cells/vial
194912	194912	D-HLF-As – Diseased Human Lung Fibroblast Cells – Asthma	Cryopreserved, in FGM™ 2 BulletKit™	≥500,000 cells/vial
194843	194843	D-HLF-CF – Diseased Human Lung Fibroblasts – Cystic Fibrosis	Cryopreserved, in FGM™ 2 BulletKit™	≥500,000 cells/vial
195277	195277	D-HLF-COPD – Diseased Human Lung Fibroblast Cell – COPD	Cryopreserved, in FGM™ 2 BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

NOTE: Normal cell media is recommended for related disease cell types.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3118	CC-3118	SAGM™ Small Airway Epithelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3119	CC-3119	SABM™ Small Airway Epithelial Cell Basal Medium		500 mL
CC-4124	CC-4124	SAGM™ Human Small Airway Epithelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3170	CC-3170	BEGM™ Bronchial Epithelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit, serum-free	Kit
CC-3171	CC-3171	BEBM™ Bronchial Epithelial Cell Basal Medium		500 mL
CC-4175	CC-4175	BEGM™ Bronchial Epithelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
193514	193514	B-ALI™ Bronchial Air Liquid Interface Medium BulletKit™	Includes growth basal medium, differentiation basal media and SingleQuots™ Kit, only sold as BulletKit™ Medium	Kit
CC-4539	CC-4539	S-ALI™ Small Airway Air Liquid Interface Medium BulletKit™	Includes growth basal medium, differentiation basal media and SingleQuots™ Kit, only sold as BulletKit™ Medium	Kit
CC-3132	CC-3132	FGM™ 2 Fibroblast Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3131	CC-3131	FBM™ Fibroblast Basal Medium		500 mL
CC-4126	CC-4126	FGM™ 2 Fibroblast Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3124	CC-3124	EGM™ Endothelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3121	CC-3121	EBM™ Endothelial Cell Basal Medium		500 mL
CC-4133	CC-4133	EGM™ Endothelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3162	CC-3162	EGM™ 2 Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit

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Ordering Information – Media

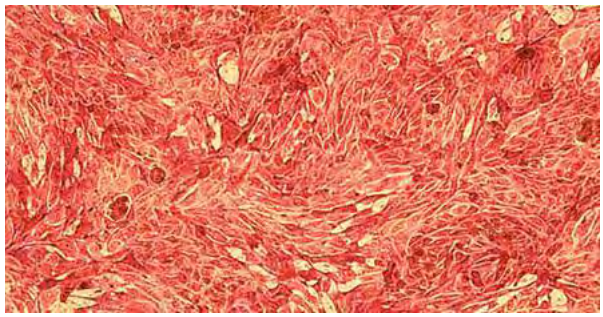
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3156	CC-3156	EBM™ 2 Endothelial Cell Basal Medium-2		500 mL
CC-4176	CC-4176	EGM™ 2 Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3182	CC-3182	SmGM™ 2 Smooth Muscle Cell Growth Medium -2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3181	CC-3181	SmBM™ Smooth Muscle Cell Basal Medium		500 mL
CC-4149	CC-4149	SmGM™ 2 Smooth Muscle Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–409.

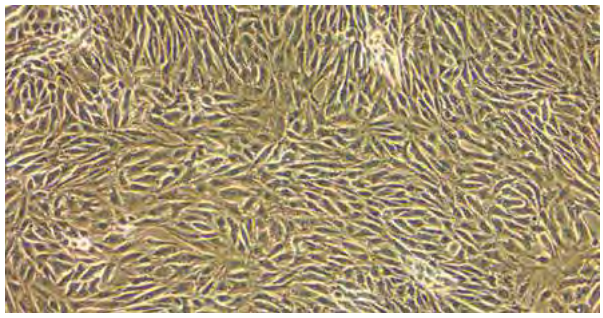
Related Products	Page
CytoSMART™ System	252
Nucleofector™ Kits for Primary Mammalian Endothelial Cells	207
Nucleofector™ Kits for Human Bronchial Epithelial Cells	208
Nucleofector™ Kits for Primary Mammalian Epithelial Cells	210
Nucleofector™ Kits for Mammalian Fibroblasts	213
Nucleofector™ Kits for Primary Mammalian Smooth Muscle Cells	218
RAFT™ 3D Culture System	256

Renal Cells and Media

Renal cells are found in the kidneys. They eliminate waste products and modulate electrolytes, pH and blood plasma volume.



RPTEC – Stained positive for g-GTP



RPTEC–100% confluency

Source

– Human kidney tissue layers specific to the designated cell type; epithelial (a mixture of cortex and glomerular), cortical epithelial (a mixture of RPTEC and distal tubule), proximal tubule epithelial (proximal tubule), and mesangial cells (renal glomerulus and modified SMC between capillaries)

Applications

Our renal proximal tubule cells are available from normal or Type 2 diabetic donors.

– Physiology
– Cancer research
– Cytokine production
– Cellular function differentiation
– Glomerulonephritis
– Prostaglandin activity
– Toxicology
– Phagocytosis of immune complexes

Cell Testing and Specifications

– **RPTEC** – Test positive for γ-GTP
– **NHMC** – Test positive for fibronectin and negative for cytokeratin 19 and von Willebrand Factor/Factor VIII
– **HRE cells** – Stain positive for pan cytokeratin
– **HRCE** – Stain positive for cytokeratin
– **All cell types** – Are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
RPTEC	Proximal tubule	REGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm^2	5 to 9 days
HRCE	Cortical epithelial	REGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm^2	5 to 9 days
HRE	Renal epithelial	REGM™ BulletKit™	1st passage	2nd passage	2,500 cells/cm^2	5 to 9 days
NHMC	Mesangial cells	MsGM™ BulletKit™	3rd passage	4th passage	3,500 cells/cm^2	5 to 9 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2554	CC-2554	HRCE – Human Renal Cortical Epithelial Cells	Cryopreserved, in REGM™ BulletKit™	≥500,000 cells/vial
CC-2556	CC-2556	HRE – Human Renal Epithelial Cells	Cryopreserved, in REGM™ BulletKit™	≥500,000 cells/vial
CC-2559	CC-2559	NHMC – Normal Human Mesangial Cells	Cryopreserved, in MsGM™ BulletKit™	≥500,000 cells/vial
CC-2553	CC-2553	RPTEC – Human Renal Proximal Tubule Epithelial Cells	Cryopreserved, in REGM™ BulletKit™	≥500,000 cells/vial
CC-2925	CC-2925	D-RPTEC – Diseased Human Renal Proximal Tubule Epithelial Cells – Diabetes Type II	Cryopreserved, in REGM™ BulletKit™	≥500,000 cells/vial

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Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-4127	CC-4127	REGM™ Renal Epithelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3190	CC-3190	REGM™ Renal Epithelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3191	CC-3191	REBM™ Renal Epithelial Cell Basal Medium		500 mL
CC-4146	CC-4146	MsGM™ Mesangial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3146	CC-3146	MsGM™ Mesangial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3147	CC-3147	MsBM™ Mesangial Cell Basal Medium		500 mL
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–406.

Related Products	Page
Cell Culture Reagents	146
CytoSMART™ System	252
Nucleofector™ Kits for Primary Mammalian Epithelial Cells	210

Reproductive Cells and Media

The human reproductive system is made up of very diverse organs which work together for the purpose of reproduction. Both male and female reproductive cells are available for the study of reproductive science and certain gender related diseases or disorders.

Source

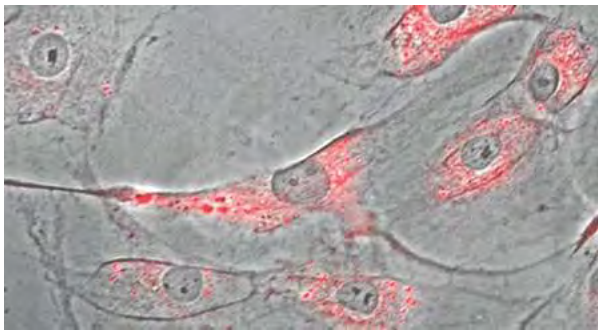
– Human male and female reproductive systems including prostate, testes, and uterine tissue

Applications

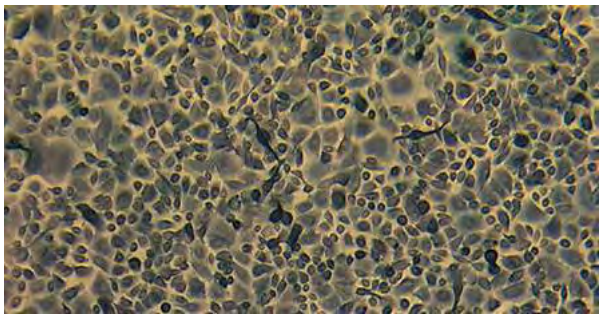
– Physiology
– Cancer research
– Toxicology
– Toxic Shock Syndrome
– Drug discovery
– Procreation research
– Male infertility
– Human papillomavirus

Cell Testing and Specifications

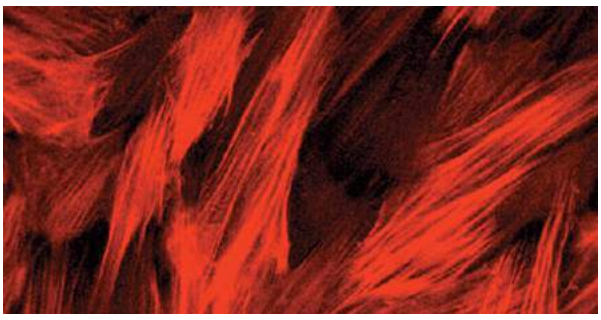
– **Prostate epithelial cells** – Test positive for cytokeratin (clone 8.13), prostate stromal cells test positive for vimentin and negative for pan cytokeratin. Both epithelial and stromal cell types are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
– **Prostate smooth muscle cells** – Stain positive for α-actin and are guaranteed to 10 population doublings when using Clonetics™ Media and Reagents
– **Uterine smooth muscle cells** – Stain positive for α-actin and negative for von Willebrand Factor after differentiation
– **Uterine MV endothelial cells** – Stain positive for acetylated low density lipoprotein uptake and for von Willebrand Factor Expression/Factor VIII, and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
– **Sertoli cells** – Test ≥70% positive for GATA-4 and Sox-9 by FACS analysis



Sertoli cell with intracellular lipid droplets stained with AdipoRed™ Assay Reagent



PrEC – Peroxidase stain for cytokeratin, clone 8.13



UtSMC – Stained for smooth muscle actin (red)

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
UtMVEC-Myo	Uterine microvascular	EGM™-MV BulletKit™	3rd passage	4th or 5th passage	5,000 cells/cm^2	5 to 9 days
UtSMC	Uterine smooth muscle	SmGM™ 2 BulletKit™	3rd passage	4th or 5th passage	3,500 cells/cm^2	6 to 10 days
UASMC	Umbilical artery	SmGM™ 2 BulletKit™	3rd passage	4th or 5th passage	3,500 cells/cm^2	6 to 10 days
PrEC	Prostate epithelial	PrEGM™ BulletKit™	1st or 2nd passage	2nd or 3rd passage	2,500 cells/cm^2	6 to 9 days
PrSC	Prostate stromal	SCGM™ BulletKit™	3rd or 4th passage	4th or 5th passage	3,500 cells/cm^2	6 to 9 days
PrSMC	Prostate smooth muscle	SmGM™ 2 BulletKit™	2nd or 3rd passage	3rd or 4th passage	3,500 cells/cm^2	6 to 9 days
HSeC	Sertoli cells	DMEM:F12 + 5% FBS	2nd passage	3rd passage	450-500 cells/cm^2	7 to 10 days

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Reproductive Cells and Media

Continued

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2562	CC-2562	UtSMC – Human Uterine Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2564	CC-2564	UtMVEC-Myo – Human Uterine Microvascular Endothelial Cells	Cryopreserved, in EGM™ 2MV BulletKit™	≥500,000 cells/vial
CC-2579	CC-2579	UASMC – Human Umbilical Artery Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2587	CC-2587	PrSMC – Human Prostate Smooth Muscle Cells	Cryopreserved, in SmGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2508	CC-2508	PrSC – Human Prostate Stromal Cells	Cryopreserved, in SCGM™ BulletKit™	≥500,000 cells/vial
CC-2555	CC-2555	PrEC – Human Prostate Epithelial Cells	Cryopreserved, in PrEGM™ BulletKit™	≥500,000 cells/vial
MM-HSE-2305	MM-HSE-2305	HSeC – Human Sertoli Cells	Cryopreserved	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Ordering Information – Media

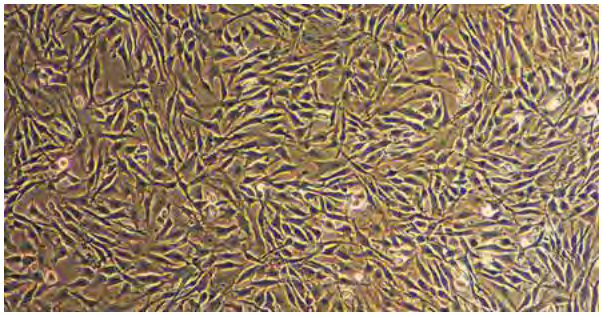
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3156	CC-3156	EBM™ 2 Endothelial Cell Basal Medium-2		500 mL
CC-3202	CC-3202	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4147	CC-4147	EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
192060	192060	KGM™ Gold Keratinocyte Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
192151	192151	KBM™ Gold Keratinocyte Basal Medium		500 mL
192152	192152	KGM™ Gold Keratinocyte Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3166	CC-3166	PrEGM™ Prostate Epithelial Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3165	CC-3165	PrEBM™ Prostate Epithelial Cell Basal Medium		500 mL
CC-4177	CC-4177	PrEGM™ Prostate Epithelial Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3205	CC-3205	SCGM™ Stromal Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3204	CC-3204	SCBM™ Stromal Cell Basal Medium		500 mL
CC-4181	CC-4181	SCGM™ Stromal Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–409.

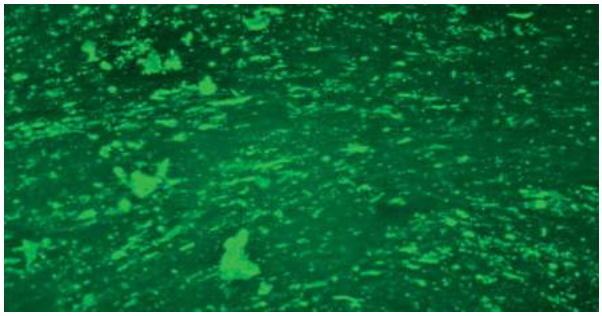
Related Products	Page
CytoSMART™ System	252
Nucleofector™ Kits for Primary Mammalian Epithelial Cells	210
Nucleofector™ Kits for Primary Mammalian Smooth Muscle Cells	218

Skeletal and Connective Tissue Cells and Media

Skeletal cells provide primary structural support as bone. Osteoblasts produce bone matrix and prime it for mineralization. Chondrocytes produce and maintain extracellular cartilage matrix. Cartilage provides joint cushioning and facilitates joint articulation. Fibroblasts are found in the stroma of tissue, where they play several important roles, such as manufacturing growth factors and protein fibers.



NHAC-kn undifferentiated 100% confluent



Day 21 Differentiated NHOst stained with OsteoImage™ Assay Kit

Source

– Human osteoblasts are sourced from spongy bone tissue, and human articular chondrocytes are isolated from the knee joint. Fibroblasts are sourced from the periodontal ligament

Applications

– Physiology
– Joint degeneration
– Fibrosis
– Joint replacement
– Bone repair
– Bone formation
– Bone disease
– Osteoporosis

Cell Testing and Specifications

– **Human articular chondrocytes** – Test positive for type II collagen and sulfated proteoglycans after differentiation and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
– **Human osteoblasts** – Test positive for alkaline phosphatase and bone mineralization and are guaranteed through 10 population doublings when using Clonetics™ Media and Reagents
– **Periodontal ligament fibroblasts** – Stain negative for pan cytokeratin and are guaranteed through 10 population doublings when using Clonetics™ Media and Reagents

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
NHOst	Osteoblasts	OGM™ BulletKit™	2nd or 3rd passage	3rd or 4th passage	5,000 cells/cm^2	6 to 9 days
NHAC-kn	Articular chondrocytes, knee	CDM™ BulletKit™	2nd passage	3rd passage	10,000 cells/cm^2	4 to 9 days
HPdLF	Periodontal ligament fibroblasts	SCGM™ BulletKit™	2nd passage	3rd passage	3,500 cells/cm^2	6 to 9 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2538	CC-2538	NHOst – Normal Human Osteoblasts	Cryopreserved, in OGM™ BulletKit™	≥500,000 cells/vial
CC-2550	CC-2550	NHAC-kn – Human Articular Chondrocytes – Knee	Cryopreserved, in CGM™ BulletKit™	≥750,000 cells/vial
CC-7049	CC-7049	HPdLF – Human Periodontal Ligament Fibroblasts	Cryopreserved, in SCGM™ BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

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Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3225	CC-3225	CDM™ Chondrocyte Differentiation Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3217	CC-3217	CBM™ Chondrocyte Basal Medium		500 mL
CC-3226	CC-3226	CDM™ Chondrocyte Differentiation Basal Medium		250 mL
CC-4408	CC-4408	CDM™ Chondrocyte Differentiation Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3216	CC-3216	CGM™ Chondrocyte Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4409	CC-4409	CGM™ Chondrocyte Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3207	CC-3207	OGM™ Osteoblast Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3208	CC-3208	OBM™ Osteoblast Basal Medium		500 mL
CC-4194	CC-4194	OGM™ Osteoblast Growth Medium Differentiation SingleQuots™ Supplements and Growth Factors	To promote differentiation	Kit
CC-4193	CC-4193	OGM™ Osteoblast Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3205	CC-3205	SCGM™ Stromal Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3204	CC-3204	SCBM™ Stromal Cell Basal Medium		500 mL
CC-4181	CC-4181	SCGM™ Stromal Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–409.

Ordering Information – Reagents

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Additional reagents required to culture chondrocytes				
CC-3233	CC-3233	Chondrocyte ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL
CC-3235	CC-3235	Calcium Chloride Solution ($CaCl_2$)		500 mL
CC-4398	CC-4398	Ascorbic Acid	25.5 mg/mL	0.5 mL
PT-4124	PT-4124	rhTGF-ß3 for chondrocyte re-differentiation		2 µg

Related Products	Page
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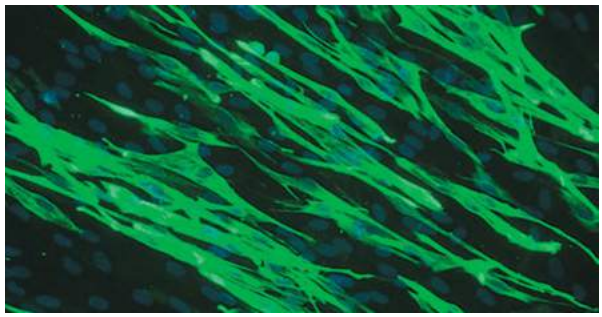
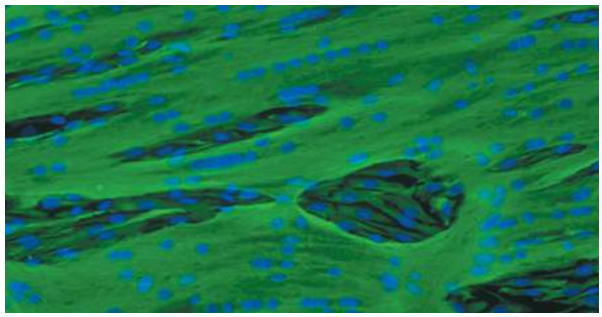
Human Osteoclast Precursors	25
Nucleofector™ Kits for Human Chondroycytes	200
OsteoImage™ Mineralization Assay	275
Rat Osteoblasts	98

Skeletal Muscle Cells and Media

Skeletal muscle cells form the striated muscles that attach to bones in the skeletal system to control body movement. Skeletal muscle myoblasts are progenitor cells that give rise to muscle cells.



Differentiated SkMC stained positive for Desmin (green) and counter-stained with DAPI (blue)



Differentiated HSMM stained positive for Desmin (green) and counter-stained with DAPI (blue)

Source

- Human skeletal muscle cells are isolated from the upper arm or upper leg, and human skeletal muscle myoblasts are isolated from post-gestational tissue, usually from quadriceps or psoas tissue

Applications

- Our human skeletal muscle myoblasts are available from normal, Type I, or Type II diabetic donors.
- Gene expression
- Receptor mediated function
- Differentiation
- Neuromuscular disease research
- Ion transport
- Diabetes
- Myopathy

Cell Testing and Specifications

- **Human skeletal muscle cells** – Test positive for desmin following differentiation and are guaranteed through 15 population doublings when using Clonetics™ Media and Reagents
- **Human skeletal muscle myoblasts** – Test positive for desmin as differentiated HSMM myotubes, when differentiated they form multinucleated myotubes in serum-poor media, or approaching confluence. Are guaranteed through 10 population doublings with normal cells when using Clonetics™ Media and Reagents

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
HSMM	Muscle myoblasts	SkGM™ 2 BulletKit™	2nd passage	3rd passage	3,500 cells/cm^2	5 to 9 days
SkMC	Skeletal muscle	SkGM™ BulletKit™	2nd passage	3rd passage	3,500 cells/cm^2	6 to 10 days

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Skeletal Muscle Cells and Media

Continued

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Normal Cells				
CC-2561	CC-2561	SkMC – Human Skeletal Muscle Cells	Cryopreserved, in SkGM™ BulletKit™	≥500,000 cells/vial
CC-2580	CC-2580	HSMM – Human Skeletal Muscle Myoblasts	Cryopreserved, in SKGM™ 2 BulletKit™	≥500,000 cells/vial
Diseased Cells				
CC-2900	CC-2900	D-HSMM – Diseased Human Skeletal Muscle Myoblasts – Diabetes Type I	Cryopreserved, in SkGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2901	CC-2901	D-HSMM – Diseased Human Skeletal Muscle Myoblasts – Diabetes Type II	Cryopreserved, in SkGM™ 2 BulletKit™	≥500,000 cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

NOTE: Normal cell media is recommended for related disease cell types.

Ordering Information – Media

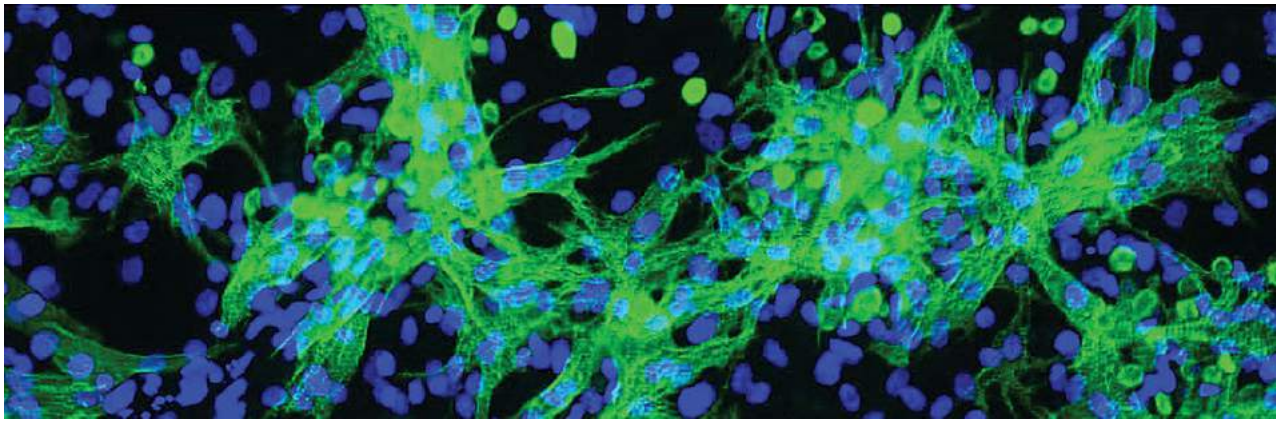
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2900	CC-2900	D-HSMM – Diseased Human Skeletal Muscle Myoblasts – Diabetes Type I	Cryopreserved, in SKGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2901	CC-2901	D-HSMM – Diseased Human Skeletal Muscle Myoblasts – Diabetes Type II	Cryopreserved, in SKGM™ 2 BulletKit™	≥500,000 cells/vial
CC-2561	CC-2561	SkMC – Human Skeletal Muscle Cells	Cryopreserved, in SkGM™ BulletKit™	≥500,000 cells/vial
CC-2580	CC-2580	HSMM – Human Skeletal Muscle Myoblasts	Cryopreserved, in SKGM™ 2 BulletKit™	≥500,000 cells/vial
CC-3160	CC-3160	SkGM™ Skeletal Muscle Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3161	CC-3161	SkBM™ Skeletal Muscle Cell Basal Medium		500 mL
CC-3244	CC-3244	SKGM™ 2 Skeletal Muscle Cell Growth Medium-2 SingleQuots™ Supplements and Growth Factors		Kit
CC-3245	CC-3245	SKGM™ 2 Skeletal Muscle Cell Growth Medium-2 BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3246	CC-3246	SKBM™ 2 Skeletal Muscle Cell Basal Medium-2		500 mL
CC-4139	CC-4139	SkGM™ Skeletal Muscle Cell Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
17-512F	BE17-512F	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) without calcium or magnesium	500 mL
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–409.

Related Products	Page
CytoSMART™ System	252

Notes

Clonetics™ Animal Primary Cells and Media



Clonetics™ Animal Primary Cells and Media

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Introduction

Clonetics™ Animal Primary Cells are provided with the same quality standards as the Clonetics™ Human Cell Products. All cells are performance tested and test negative for mycoplasma, bacteria, yeast and fungi. Clonetics™ Cells are guaranteed to perform as indicated when used with Clonetics™ Cells, Media and Reagents. Immuno and special staining protocols, as well as characteristic morphology, are used to characterize the cells and assure they are the designated type. A Certificate of Analysis is available for each cell type and lot.

General Cell and Media Information

- Clonetics™ Cells are guaranteed to perform to our release criteria if cultured in our appropriate media
- Where possible, the media systems are offered as BulletKit™ Products (basal medium and separately packaged growth factors) to provide the flexibility to manipulate media components specific to your application

General Ordering and Shipping Information

Cryopreserved cells and media products are normally shipped Monday – Thursday for next day delivery. Saturday and Monday deliveries are available upon special request.

Other cell types may be available upon request. Plate orders are an additional passage and take an extra week.

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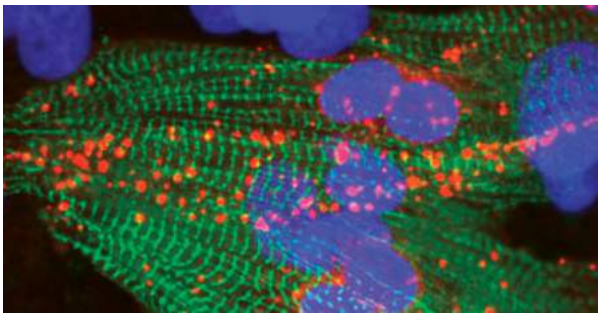
Cardiac Cells and Media

Cardiac cells are used to study the functions of the vascular system and general pathophysiology of the cardiovascular system.



Rat cardiac myocytes stained for alpha actinin (green), connexin 43 (red), and DAPI (blue)

Rat AoSMC cells at passage 4 stained for α-smooth muscle actin (red)

Source

- Rat cardiac myocytes isolated from neonatal Sprague Dawley rat hearts (ventricular tissue)
- Rat aortic smooth muscle cells are isolated from the aorta of 150–200 gram adult male Sprague-Dawley rats

Applications

- Arrhythmia
- Heart failure
- Angiogenesis
- Vascular research
- Cardiomyopathy
- Preventative cardiology
- Artherosclerosis

Cell Testing and Specifications

- **Rat cardiac myocytes** – Each vial contains approximately 4 million viable cells at ≥85% purity. When thawed and cultured, you will obtain ≥80% viability, with excellent morphology and connectivity, and cells will display beating at 24 hours in culture. Each lot tests positive for functional syncytium formation and stain positive for actinin. Cell function guaranteed when using Clonetics™ Media and Reagents. Primary cardiac myocyte cells need an appropriate substrate to adhere and survive – the preferred substrate is nitrocellulose
- **Rat aortic smooth muscle cells** – Stain ≥95% positive for α-actin and negative for VE cadherin and are guaranteed through 12 population doublings when using the recommended media and reagents

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
R-CM	Rat cardiac myocytes	RCGM™ BulletKit™	Immediate	n/a	see below*	n/a
R-ASM	Rat aortic smooth muscle	DMEM:F12 + supplements	2nd passage	3rd passage	5,000 cells/cm^2	5 to 7 days

*1 mL cell suspension + 9 mL media in 24-well plate (1 mL/well) or 1 mL cell suspension + 4.3 mL media in 96-well plate (200 µL/well)

Ordering information on the next page.

Cardiac Cells and Media

Continued

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
R-ASM-580	R-ASM-580	R-AoSM – Rat Aortic Smooth Muscle Cells	Cryopreserved	≥500,000 cells/vial
R-CM-561	R-CM-561	R-CM Rat Cardiac Myocytes	Cryopreserved	≥4 million cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-4515	CC-4515	rCGM Rat Cardiac Myocyte Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3275	CC-3275	rCBM Rat Cardiac Myocyte Basal Medium		200 mL
CC-4516	CC-4516	RCGM™ Rat Cardiac Myocyte Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-4519	CC-4519	5-Bromo-2'Deoxyuridine		Vial
BE04-687Q	BE04-687Q	Dulbecco's Modified Eagle Medium:F12 (DMEM:F12)	1:1 mixture with 3.151 g/L glucose, with UltraGlutamine™ I, without HEPES	1 L
CC-4083	CC-4083	Gentamicin sulfate / Amphotericin (GA-1000)		5 mL
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

Related Products	Page
CytoSMART™ System	252
Human Cardiac Cells	58,64
Nucleofector™ Kits for Human Chondrocytes	200

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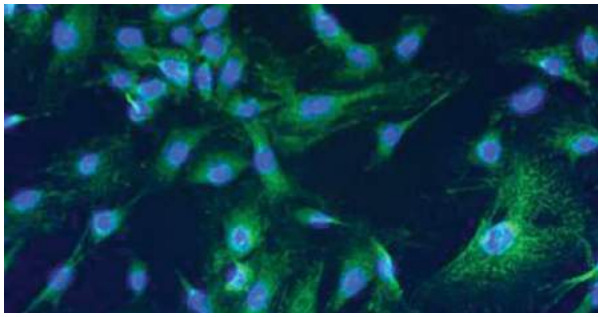
Fibroblasts Cells and Media

Mouse embryonic fibroblasts are often used as a feeder layer to culture ES cells. They provide both a substrate for the ES cells to grow on and secrete growth factors necessary for ES cells to maintain pluripotency.



MEF stained with Vimentin (green) at day 3 of second passage post-thaw and counterstained with DAPI (blue)

Source

– Mouse primary embryonic fibroblasts dissociated from day 14 and 15 post-coitus CD-1 mouse embryos, expanded and then cryopreserved as frozen primaries. They have not been treated with mitomycin-C

Applications

– Embryonic stem cell research
– Feeder layer for other cell types

Cell Testing and Specifications

– **Mouse primary embryonic fibroblasts** – Stain positive for vimentin expression, are guaranteed for five population doublings, and display morphologic and growth properties equivalent to freshly prepared cells when approved media and supplements are used

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
MEF	Mouse embryonic fibroblasts	DMEM high glucose containing 10% FBS	1st passage	2nd passage	8,000 cells/cm^2	5 to 7 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
M-FB-481	M-FB-481	MEF – Mouse Embryonic Fibroblasts	Cryopreserved	≥2 million cells/vial

For proliferating cells and cell pellets in RNALater® contact Customer Service for order placement.

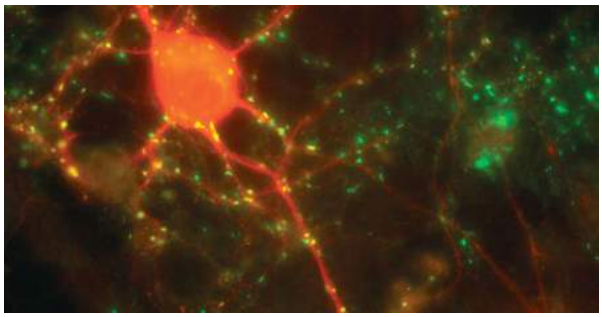
Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
12-604F	BE12-604F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, with L-Glutamine	500 mL

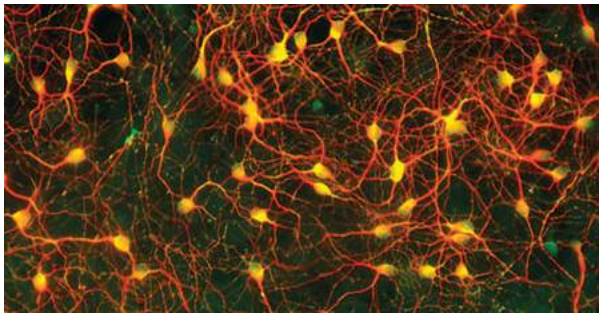
Related Products	Page
CytoSMART™ System	252
Nucleofector™ Kits for Mouse Embryonic Fibroblasts	212

Neural Cells and Media

Frozen primary neuronal cells expedite and simplify cell culture research because they can be thawed and cultured on demand to obtain high quality and high yield cultures of dissociated primary neurons.
Shipped overnight to your laboratory, these high quality, cryopreserved, dissociated primary cells represent a cost effective way to do neuronal primary cell culture, eliminating costly and time consuming animal care requirements and allowing you to control the experimental/assay timetable.
Cryopreserved neuronal cells can be shipped anywhere and used any time.



Cortical and striatal rat neurons cells were thawed, co-cultured 21 days, and immunofluorescently stained with anti- vesicular GABA transporter (vGAT) (green) and anti-dopamine receptor protein (DARPP) (red).



Rat cortical neuronal cells were thawed, cultured 14 days, and immunofluorescently stained with anti-PGP 9.5 and anti β-tubulin.

Source

- Primary rat neurons isolated from rat brain as a native mix of high quality primary embryonic brain neuronal cells (including glia)
- Rat astrocytes are obtained from rat brain, passaged once, and cryopreserved
- Primary mouse neurons and astrocytes are isolated from two different mouse strains, C57 Black and CD1

Applications

- Transfection
- Evaluation of electrophysiological properties, neurotransmitters, receptor function
- Research typical inhibitory or excitatory ion-channels
- Receptor signaling research
- Intracellular transport studies
- Neurotoxicity research

Cell Testing and Specifications

- **Rat neurons** – Each vial of rat neuronal cells is guaranteed to be mycoplasma and bacteria free. Additional molecular and immunochemical testing (PGP and Tuj) for quality is done following conditions that mimic shipping (specific cell types may vary). Prior to cryopreservation, each vial (1 mL) of cortical and striatal neurons contain approximately 4 million viable cells. Each vial (0.25 mL) of hippocampus neurons contain approximately 1 million viable cells
- **Rat astrocytes** – Are offerd isolated from the hippocampus, cortex, or striatum of the brain or as a mixed population isolated from the hippocampus, cortex, and striatum of the brain. These astrocytes are passaged once and cryopreserved. Each vial (1.0 mL) of rat astrocytes contains approximately 1 million viable cells. Following confluence, the astrocytes can be harvested once for re-plating. Each vial of astrocytes is guaranteed mycoplasma and bacteria free. Astrocytes are batch-tested for growth characteristics and morphology (GFAP)
- **Mouse neurons** – Are available isolated from the hippocampus, cortex, or striatum of the brain. These neurons are cryopreserved immediately after isolation without culturing. Each vial (1.0 mL) of mouse cortex or striatum neurons contain approximately 4 million viable cells. Each vial (0.25 mL) of mouse hippocampal neurons contain approximately 1 million viable cells. Each vial of neurons is guaranteed mycoplasma and bacteria free. Additional molecular and immunochemical testing for specific neuronal markers is also performed depending on cell type
- **Mouse astrocytes** – Are a mixed population isolated from the hippocampus, cortex, and striatum of the brain. These astrocytes are passaged once and cryopreserved. Each vial (0.5 mL) of mouse astrocytes contains approximately 1 million viable cells. Following confluence, the astrocytes can be harvested once for re-plating. Each vial of astrocytes is guaranteed mycoplasma and bacteria free. Astrocytes are batch-tested for growth characteristics and morphology (GFAP)

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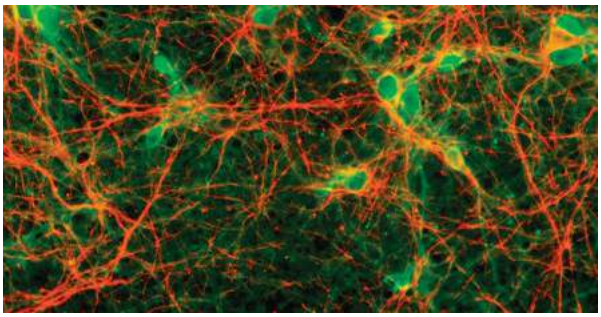
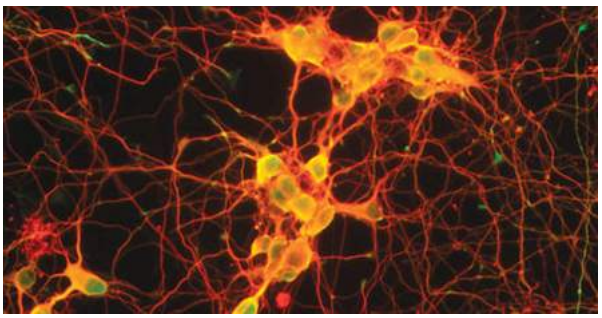
Neural Cells and Media

Continued



Immunofluorescence image of cryopreserved rat cortical cells thawed and cultured 21 days stained with anti-PGP 9.5 and anti- neurofilament.



Cryopreserved mouse cortical neuronal cells were thawed and cultured 12 days, then immunofluorescently stained with anti-PGP 9.5 and anti-β-tubulin.

Cell Type	Description	Recommended Media	Cryopreserved Cells	Culture Time
R-Cx	Rat brain cortex neurons	PNGM™ BulletKit™	Immediate	14–21 days
R-Hi	Rat brain hippocampus neurons	PNGM™ BulletKit™	Immediate	14–21 days
R-Cp	Rat brain striatum neurons	PNGM™ BulletKit™	Immediate	14–21 days
R-Drg	Rat dorsal root ganglion neurons	PNGM™ BulletKit™	Immediate	14–21 days
R-eDrg	Embryonic rat dorsal root ganglion neurons	PNGM™ BulletKit™	Immediate	14–21 days
R-Cb	Rat cerebellar neurons	PNGM™-A BulletKit™	Immediate	14–21 days
R-HTh	Rat brain hypothalamic neurons	PNGM™ BulletKit™	Immediate	14–21 days
R-CxAs	Rat brain cortex astrocytes	AGM™ BulletKit™	Primary passage	14–21 days
R-HiAs	Rat brain hippocampus astrocytes	AGM™ BulletKit™	Primary passage	14–21 days
R-CpAs	Rat brain striatum astrocytes	AGM™ BulletKit™	Primary passage	14–21 days
R-AsM	Rat brain Cx-Hi-Cp mix astrocytes	AGM™ BulletKit™	Primary passage	14–21 days
R-G	Rat microglia	DMEM high glucose containing 10% FBS	Immediate	7+ days
M-Cx	Mouse brain cortex neurons	PNGM™ BulletKit™	Immediate	14–21 days
M-Cp	Mouse brain striatum neurons	PNGM™ BulletKit™	Immediate	14–21 days
M-Hi	Mouse brain hippocampus neurons	PNGM™ BulletKit™	Immediate	14–21 days
M-AsM	Mouse brain mixed astrocytes	AGM™ BulletKit™	Primary passage	21+ days

Ordering information on the next page.

Neural Cells and Media

Continued

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
M-AsM-430	M-AsM-430	M-AsM – Mouse CD1 Brain Mixed Astrocytes	Cryopreserved, 0.5 mL	≥1 million cells/vial
M-AsM-330	M-AsM-330	M-AsM – Mouse CD57 Brain Mixed Astrocytes	Cryopreserved, 0.5 mL	≥1 million cells/vial
M-Cp-302	M-Cp-302	M-Cp – Mouse C57 Brain Striatum Neurons	Cryopreserved, 1.0 mL	≥4 million cells/vial
M-Cp-402	M-Cp-402	M-Cp – Mouse CD1 Brain Striatum Neurons	Cryopreserved, 1.0 mL	≥4 million cells/vial
M-Cx-300	M-Cx-300	M-Cx – Mouse C57 Brain Cortex Neurons	Cryopreserved, 1.0 mL	≥4 million cells/vial
M-Cx-400	M-Cx-400	M-Cx – Mouse CD1 Brain Cortex Neurons	Cryopreserved, 1.0 mL	≥4 million cells/vial
M-Hi-401	M-Hi-401	M-Hi – Mouse Brain Hippocampus Neurons	Cryopreserved, 0.25 mL	≥1 million cells/vial
R-AsM-530	R-AsM-530	R-AsM – Rat Brain Cx-Hi-Cp Mix Astrocytes	Cryopreserved, 1.0 mL	≥1 million cells/vial
R-Cb-503	R-Cb-503	R-Cb – Rat Cerebellar Neurons	Cryopreserved, granule cells, 1.0 mL	≥4 million cells/vial
R-Cp-502	R-Cp-502	R-Cp – Rat Brain Striatum Neurons	Cryopreserved, 1.0 mL	≥4 million cells/vial
R-CpAs-522	R-CpAs-522	R-CpAs – Rat Brain Striatum Astrocytes	Cryopreserved, 1.0 mL	≥1 million cells/vial
R-Cx-500	R-Cx-500	R-Cx – Rat Brain Cortex Neurons	Cryopreserved, 1.0 mL	≥4 million cells/vial
R-CxAs-520	R-CxAs-520	R-Cx-As – Rat Brain Cortex Astrocytes	Cryopreserved, 1.0 mL	≥1 million cells/vial
R-Drg-505	R-Drg-505	R-DRG – Rat Dorsal Root Ganglion Neurons	Cryopreserved, 0.25 mL	≥200,000 cells/vial
R-eDRG-515	R-eDRG-515	R-eDRG – Rat Dorsal Root Ganglion Neurons – Embryonic	Cryopreserved, 0.25 mL	≥1 million cells/vial
R-G-535	R-G-535	R-G – Rat Microglia	Cryopreserved, 0.25 mL	≥2 million cells/vial
R-Hi-501	R-Hi-501	R-Hi – Rat Brain Hippocampus Neurons	Cryopreserved, 0.25 mL	≥1 million cells/vial
R-HiAs-521	R-HiAs-521	R-HiAs – Rat Brain Hippocampus Astrocytes	Cryopreserved, 1.0 mL	≥1 million cells/vial
R-Hth-507	R-Hth-507	R-Hth – Rat Brain Hypothalamus Neurons	Cryopreserved, 0.5 mL	≥2 million cells/vial

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-4461	CC-4461	PNGM™ Primary Neuron Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3256	CC-3256	PNBM™ Primary Neuron Basal Medium		200 mL
CC-4462	CC-4462	PNGM™ Primary Neuron Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-4512	CC-4512	PNGM™ A Primary Neuron Growth Medium – Adult BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4511	CC-4511	PNGM™-A Primary Neuron Growth Medium – Adult SingleQuots™ Supplements and Growth Factors		Kit
CC-3186	CC-3186	AGM™ Astrocyte Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-3187	CC-3187	ABM™ Astrocyte Basal Medium		200 mL
CC-4123	CC-4123	AGM™ Astrocyte Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–408.

Related Products	Page
Human Neural Cells	71
Human Neural Progenitor Cells	24
Rat Retinal Cells	97
Adherent Nucleofection	172, 184
Nucleofector™ Kits for Primary Neural Cells	219, 223

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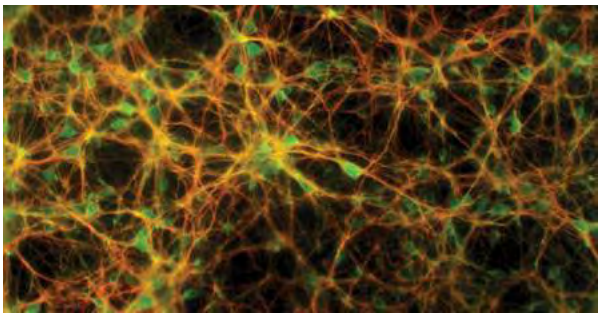
Ocular Cells and Media

The vertebrate retina is a light sensitive tissue lining the inner surface of the eye. Light strikes the retina, creates an image and initiates a cascade of chemical and electrical events that ultimately trigger nerve impulses. These impulses are sent to visual centers of the brain through the fibers of the optic nerve.



Rat retinal cells stained for neuron specific class III β-tubulin (Tuj-1) and neuronal protein gene product (PGP 9.5)

Source

– Rat retinal cells isolated from neonatal (day 3–4) Sprague-Dawley rats and comprised of the seven cell types normally found in retina. They are prepared by dissection/dissociation without purification, cryopreserved, and are ready for immediate culture

Applications

– General ophthalmic research
– Posterior segment disease
– Neoplasms
– Cell therapies
– Toxicology and cytotoxicity
– Inflammation
– Drug delivery
– Degeneration
– Gene expression

Cell Testing and Specifications

– **Rat retinal cells** – Each lot tests negative for mycoplasma and sterility. Immunostaining for neuron specific class III β-tubulin (Tuj-1), specific neuronal protein gene product (PGP 9.5), ganglion cell marker, Thy1.1, and GFAP

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Culture Time
R-Ret	Rat retinal cells	PNGM™ BulletKit™	Immediate	n/a	14–21 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
R-ReT-508	R-ReT-508	R-Ret-Neo – Rat Retinal Cells, neonatal	Cryopreserved, 0.5 mL	≥200,000 cells/vial

Ordering Information – Media

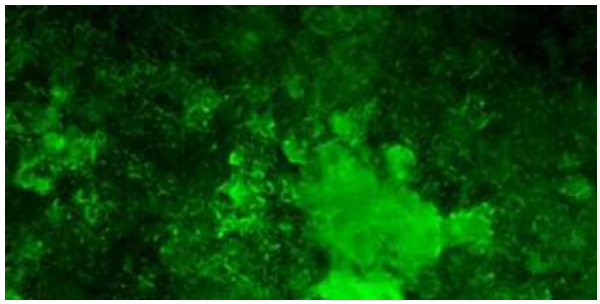
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-4461	CC-4461	PNGM™ Primary Neuron Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Kit
CC-4462	CC-4462	PNGM™ Primary Neuron Growth Medium SingleQuots™ Supplements and Growth Factors		Kit
CC-3256	CC-3256	PNBM™ Primary Neuron Basal Medium		200 mL
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each

See pages 400–408.

Related Products	Page
Human Neural Cells	71
Human Neural Progenitor Cells	24

Skeletal Cells and Media

Skeletal cells provide primary structural support as bone. Osteoblasts produce bone matrix and prime it for mineralization. Bone cells are responsible for the body's response trauma and fracture to strengthen, develop, heal, and grow bone.



Rat osteoblasts stained with OsteoImage™ Assay for mineralization at day 24

Source

– Rat calvariae osteoblasts dissociated from Sprague-Dawley rat embryos (E20, E21)

Applications

– Physiology
– Bone formation
– Joint replacement
– Bone repair
– Bone disease
– Osteoporosis

Cell Testing and Specifications

– **Rat osteoblasts** – Are cryopreserved at dissection and each vial of osteoblasts contains ≥0.35 million viable cells. This will seed into approximately three 6-well plates for mineralization studies, three T-25 flasks or one T-75 flask for proliferation studies using the recommended plating densities and medium

– **Rat osteoblasts** – Will undergo at least 12 population doublings and are tested for mineralization after differentiation. For mineralization studies, it is recommended to plate cells directly out of cyropreservation into multi-well plates. Upon inducing differentiation, cells require 3 to 5 weeks to sufficiently form mineralized nodules

Cell Type	Description	Recommended Media	Cryopreserved Cells	Proliferating Cells	Recommended Seeding Density	Time to Subculture
R-OST	Rat calvariae osteoblasts	DMEM high glucose	1st Passage	n/a	5,000–7,000 cells/cm^2	5 to 7 days

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
R-OST-583	R-OST-583	R-OST – Rat Calvariae Osteoblasts	Cryopreserved	≥500,000 cells/vial

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
12-604F	BE12-604F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, with L-Glutamine	500 mL

Related Products	Page
CytoSMART™ System	252
Human MSCs	29
Human Skeletal and Connective Tissue	84
OsteoImage™ Mineralization Assay	275

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Ordering Information – Reagents

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-5002	CC-5002	Trypsin Neutralizing Solution		100 mL
CC-5012	CC-5012	Trypsin/EDTA Solution		100 mL
CC-5022	CC-5022	HEPES Buffered Saline Solution		100 mL
CC-5024	CC-5024	HEPES Buffered Saline Solution		500 mL
CC-5034	CC-5034	ReagentPack™ Subculture Reagents	Trypsin/EDTA, trypsin neutralizing solution, and HEPES buffered saline solution	100 mL each
T100A	T100A	Retronectin® Recombinant Human Fibronectin Fragment	Recombinant human bronectin fragment CH-296 produced in *E.coli*. When coated on the surface of asks and plates, Retronectin® significantly enhances retrovirus-mediated gene transfer into mammalian cells.	0.5 mg
T100B	T100B	Retronectin® Recombinant Human Fibronectin Fragment	Recombinant human fibronectin fragment CH-296 produced in E.coli. When coated on the surface of asks and plates, Retronectin® significantly enhances retrovirus-mediated gene transfer into mammalian cells.	2.5 mg
T110A	T110A	Retronectin® Dish		10 dishes (35mm)

Additional Cell Culture Reagents can be found on pages 400–418.

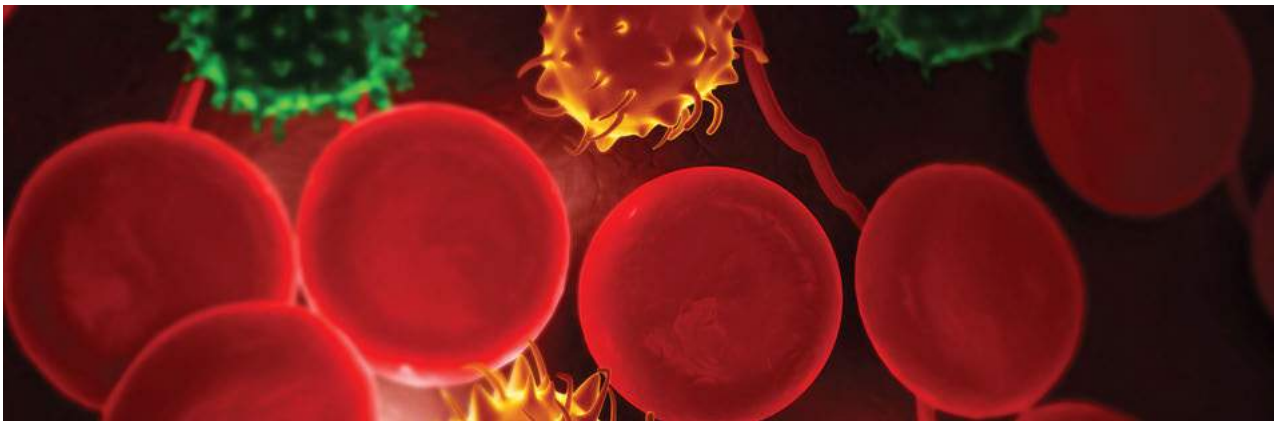
Ordering Information – Growth Factors

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-4009	CC-4009	Bovine Pituitary Extract	13 mg/mL	2 mL
CC-4068	CC-4068	hFGF – Human Fibroblastic Growth Factor	1 µg/mL	1 mL
CC-4098	CC-4098	Bovine Brain Extract	9 mg/mL	5 mL
CC-4092	CC-4092	Bovine Brain Extract	3 mg/mL	2 mL
CC-4107	CC-4107	hEGF Human Epidermal Growth Factor	3 µg/mL	0.5 mL
CC-4202	CC-4202	Calcium Chloride	300 mM	2 mL
CC-4205	CC-4205	Human Transferrin	10 mg/mL	0.5 mL
CC-4323	CC-4323	NSF-1 Neural Survival Factor-1	50X Concentration	4 mL
CC-4398	CC-4398	Ascorbic Acid	25.5 mg/mL	0.5 mL

Additional Cell Culture Reagents can be found on pages 400–418.

Poietics™ Immune Cells and Media

Leading the attack on immune cell research



Poietics™ Immune Cells and Media

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Introduction

Poietics™ Primary Human Immune Cells and Media are isolated from either peripheral blood or cord blood from healthy donors. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Poietics™ Primary Human Immune Cells purity levels are verified via flow cytometric analysis.

Poietics™ Primary Human Immune Cells and Media can be used in various immunological research applications, including:

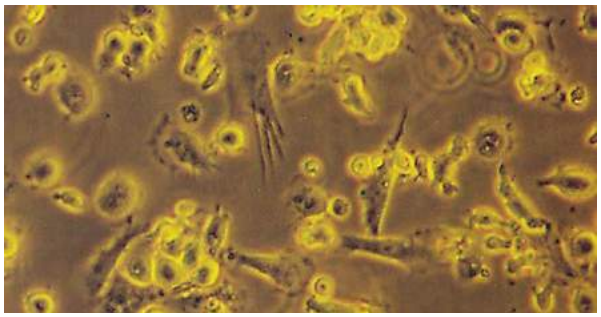
- Transplantation
- Autoimmune diseases
- HIV/AIDS
- Cellular immunity
- Immunotherapy
- Cancer
- Cell memory
- Histocompatability
- Graft-versus-host disease

Human Peripheral Blood Dendritic and Mononuclear Cells

Poietics™ Normal Human Dendritic Cells and Media Consist of:

- Cryopreserved primary derived cultures of – normal human dendritic cells derived from human peripheral blood mononuclear cells
- LGM-3™ Lymphocyte Growth Medium-3, a serum-free medium for lymphocyte cell growth

All cells are isolated from peripheral blood mononuclear cells using the Elutra® cell separation system and are quality tested for optimal performance.
Poietics™ Normal Human Dendritic Cells are tested for a panel of specific surface markers, including $CD11c^+$, $CD86^+$, $CD80^+$. $HLADR^+$, and CD14-. These cells do not proliferate and can survive up to 7 days in culture with the proper cytokines (IL-4 and GM-CSF). Poietics™ Human Peripheral Blood Mononuclear Cells, when properly stimulated, can divide in culture.



Normal human dendritic cells

Applications

- Antigen presentation and uptake
- Pharmacology
- Drug-induced immunosuppression of cytokine secretion
- Allergy research
- Wound and infection

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2701	CC-2701	NHDC – Human Dendritic Cells	Cryopreserved	≥2.5 million cells/vial
CC-2702	CC-2702	HPBMC – Human Peripheral Blood Mononuclear Cells	Cryopreserved, volume discount available	≥50 million cells/vial

Please inquire about availability of NHDC and HPBMC matched sets from the same donor

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Size
CC-3211	CC-3211	LGM™ 3 Lymphocyte Growth Medium-3	500 mL

Related Products	Page
Nucleofector™ Kits for Primary Blood Cells	189 , 199

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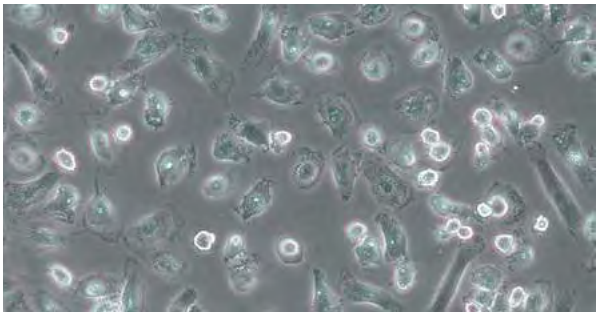
Human CD14$^+$ Monocytes and Media

Normal human monocytes are found in the circulating peripheral blood. They play an important role in host defense as circulating monocytes and differentiation into tissue macrophages, and can differentiate into dendritic cells with potent antigen-presenting capability, *in vivo* and *in vitro*.

Peripheral blood is collected from screened, healthy donors using apheresis and monocytes are isolated from this peripheral blood. The cell product, enriched for mononuclear cells, is further processed by density centrifugation to remove the remaining red cells and neutrophils. Monocytes are then isolated using positive immunomagnetic selection directed against CD14.



Human CD14$^+$ monocytes differentiating to macrophages

Media Recommendations

To maintain the monocyte phenotype, serum-containing medium is recommended (10% FBS is generally recommended). M-CSF (at 10 ng/mL) can also be added. To produce osteoblasts, both soluble RANK ligand (30 ng/mL) and M-CSF (30 ng/mL) need to be added to serum-containing medium. To produce dendritic cells, a serum-free medium, such as LGM™ 3 Lymphocyte Growth Medium-3 should be used, with the addition of GM-CSF (50 ng/mL) and IL-4 (50 ng/mL).

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
2W-400C	2W-400C	Human Peripheral Blood CD14$^+$ Monocytes	Cryopreserved	≥10 million cells/vial
2W-400B	2W-400B	Human Peripheral Blood CD14$^+$ Monocytes	Cryopreserved	≥20 million cells/vial
2W-400A	2W-400A	Human Peripheral Blood CD14$^+$ Monocytes	Cryopreserved	≥40 million cells/vial

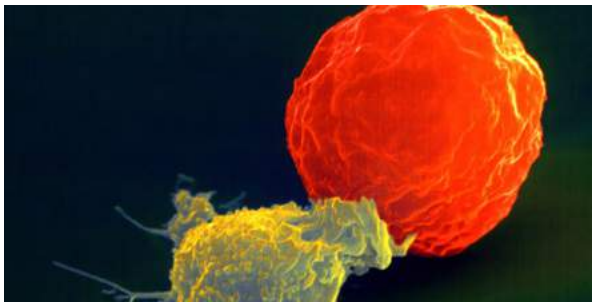
Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Size
CC-3211	CC-3211	LGM™ 3 Lymphocyte Growth Medium-3	500 mL

Related Products	Page
Nucleofector™ Kits for Human Monocytes	195

Human Natural Killer (NK) Cells

Human natural killer cells are lymphocytes of the immune system that are critical in host defense and immune regulation. Since they are part of innate immunity, they do not require sensitization for the expression of their activity. NK cells play significant roles in viral infections, autoimmunity, pregnancy, cancer, bone marrow transplantation, and more recently, adaptive immunity. NK cells are most traditionally characterized by the presence of surface marker CD56, and absence of CD3. Poietics™ Human Natural Killer Cells are isolated using positive or negative immunomagnetic selection. Purity is ≥90% via flow cytometry based on the presence of the CD56 antigen. CD16 expression is also reported. Negative selection of NK cells results in significantly greater enrichment of $CD16^+$ cells (usually >80% of total cells), than does positive selection which has much greater variability for $CD16^+$ cells. Cells isolated via positive selection are also referred to as natural killer T cells.



Human natural killer cell

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
2W-501	2W-501	NK – Human Natural Killer Cells	Cryopreserved, negative selection	≥5 million cells/vial
2W-502	2W-502	NK – Human Natural Killer Cells	Cryopreserved, positive selection	≥5 million cells/vial

Human T Cells and Media

Poietics™ Human $CD4^+$ T Cells are a type of lymphocyte that play an important role in the immune system and lead attack against infection. These cells are also referred to as helper T cells. These helper T cells orchestrate the body's response to certain pathogens, such as viruses.
Poietics™ $CD4^+$ T Cells are isolated from normal peripheral blood mononuclear cells using negative immunomagnetic selection of the CD4 antigen. Purity is ≥ 90%.

Applications
- Immunotherapy
- Inflammatory response
- Autoimmunity

Ordering Information – Cells

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
2W-200	2W-200	Human Peripheral Blood $CD4^+$ T Cells	Cryopreserved	≥10 million cells/vial

Ordering Information – Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-3211	CC-3211	LGM™ 3 Lymphocyte Growth Medium-3		500 mL

Related Products	Page
Nucleofector™ Kits for Human T Cells	197

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Introduction

Liquid Cell Culture Media

Chemically defined liquid media are used to provide nutrients for cell culture growth in research, diagnostic, and manufacturing applications. In order to meet your specific needs, our production and quality control procedures for liquid media emphasize control. Our goal is to provide you with high quality products that are consistent from batch to batch. With all of the time that you take to optimize your systems, we want to provide you with products you can trust.

All BioWhittaker™ Classical Media products are labelled for research use only and are manufactured in accordance with ISO:9001. A Device Master Record (DMR) is prepared for every liquid medium product. It defines the procedures for production from receipt of raw materials to final product release, the environmental and processing controls required, as well as product specifications, including packaging and labeling. Product manufacturing is consistent with the requirements defined in the DMR, which ensures that each lot of a product is consistent with all other lots of the same product.

Chemicals used to prepare liquid media products are purchased according to the raw material qualifications from approved suppliers. Each lot must meet established component specifications before it is released by Quality Assurance for use. We manufacture all liquid cell culture media using Water for Injection (WFI) quality water, which has been prepared by ultrafiltration, reverse osmosis, deionization, and distillation. Liquid media is sterile filtered through pharmaceutical-grade sterilizing filters. The formulations used for standard classical media products are those recommended by the Tissue Culture Association.

Quality Control

In order to maintain consistent quality in sterile cell culture media products, strict quality control of each production lot is essential. Written procedures in accordance with current Good Manufacturing Practices (cGMPs) provide quality control from start to finish for each product produced. Final product testing includes the following:

USP Sterility – Tests are performed on representative samples using the membrane filtration procedure in accordance with the US Pharmacopoeia or EP Pharmacopoeia. Culture media used are fluid thioglycollate medium (FTM) and trypticase soy broth (TSB). The test samples are filtered and the filters are immersed in FTM and TSB. TSB cultures are incubated at 22.5°C ± 2.5°C. FTM cultures are incubated at 32.5°C ± 2.5°C. The cultures are incubated for 14 days, during which they are periodically examined and sterility results are recorded.

Chemistries (pH and osmolality) – Tests are performed on representative samples from each lot using routinely calibrated equipment. Osmolality is determined by means of the highly repeatable freezing point method.

Endotoxin – Products are tested for endotoxin content using the Kinetic-QCL™ Assay. Test dilutions used are screened for inhibition and enhancement in the Kinetic-QCL™ Assay. Endotoxin levels for these products are available on Certificates of Analysis.

The combined result of all in-process monitoring and final product testing is the further assurance that each lot has been prepared not only according to approved written procedures, but has passed test criteria, and will meet design specifications. Samples from each lot of standard product are retained and stored at label temperature in the event follow up testing is required. Subsequent testing may be in response to your inquiries or for shelf life studies.

Basal Medium Eagle (BME)

A minimal medium suitable for a variety of cell types. It is a historical precursor to Minimum Essential Medium (MEM).

15°C to 30°C

Ordering Information – Basal Medium Eagle

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-132A	12-132A	Cryoprotective Freezing Medium	Without L-Glutamine, with 15% DMSO (Use 1:1 with growth medium)	15°C to 30°C	100 mL
12-105F	BE12-105F	Basal Medium Eagle (BME)	With Earle's BSS, without L-Glutamine	15°C to 30°C	500 mL

Cryoprotective Medium – see ProFreeze™-CD, non-animal origin, chemically-defined freeze medium, Cat. No. 12-769E, page 130.

For a basic procedure for cryopreservation of cells, see page 416.

Dulbecco's Modified Eagle Medium (DMEM)

DMEM is used in a wide range of mammalian cell culture applications. The high glucose version is well suited to high density suspension culture. The low glucose formula is used for adherent cells.

2°C to 8°C

		Without L-glutamine	With Sodium Pyruvate	Without Phenol Red	With HEPES	With 1.0 g/L Glucose	With 4.5 g/L Glucose	Powder	Hybridoma Screened	With UG I*
Cat. No.	Size									
12-614F	500 mL	■	■				■			
12-614Q	1 L	■	■				■			
12-604F	500 mL		■				■			
12-604Q	1 L		■				■			
BE12-604F/U1	500 mL	■	■				■			■
12-914F	500 mL	■	■				■		■	
12-917F	500 mL	■	■	■			■			
12-707F	500 mL	■	■			■				
12-708F	500 mL	■	■		■	■				
12-709F	500 mL	■	■		■		■			
12-733F	500 mL	■					■			
12-733Q	1 L	■					■			
12-741F	500 mL						■			

* With UltraGlutamine I

Dulbecco's Modified Eagle Medium (DMEM)

Continued

Ordering Information – Dulbecco's Modified Eagle Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-614F	BE12-614F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, without L-Glutamine	2°C to 8°C	500 mL
12-614Q	BE12-614Q	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, without L-Glutamine	2°C to 8°C	1 L
12-604F	BE12-604F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, with L-Glutamine	2°C to 8°C	500 mL
12-604Q	BE12-604Q	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, with L-Glutamine	2°C to 8°C	1 L
BE12-604F/U1	BE12-604F/U1	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, with UltraGlutamine™ I	2°C to 8°C	500 mL
12-914F	BE12-914F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, without L-Glutamine, hybridoma tested	2°C to 8°C	500 mL
12-917F	BE12-917F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, without L-Glutamine or phenol red	2°C to 8°C	500 mL
12-707F	BE12-707F	Dulbecco's Modified Eagle Medium (DMEM)	With 1.0 g/L glucose, without L-Glutamine	2°C to 8°C	500 mL
12-708F	BE12-708F	Dulbecco's Modified Eagle Medium (DMEM)	With 1.0 g/L glucose, without L-Glutamine, with 25 mM HEPES	2°C to 8°C	500 mL
12-709F	BE12-709F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, without L-Glutamine, with 25 mM HEPES	2°C to 8°C	500 mL
12-733F	BE12-733F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, without L-Glutamine or sodium pyruvate	2°C to 8°C	500 mL
12-733Q	12-733Q	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, without L-Glutamine or sodium pyruvate	2°C to 8°C	1 L
12-741F	BE12-741F	Dulbecco's Modified Eagle Medium (DMEM)	With 4.5 g/L glucose, with L-Glutamine, without sodium pyruvate	2°C to 8°C	500 mL

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Dulbecco's Modified Eagle Medium:F12 (DMEM:F12)

DMEM:F12 combines the richness of F12 with the higher component concentrations of DMEM. This medium is well – suited for clonal density cultures.

2°C to 8°C

Cat. no.	Size	Without L-Glutamine	With HEPES	With 3.151 g/L Glucose	Powder	With UG I*
12-719F	500 mL		■	■		
12-719Q	1 L		■	■		
BE04-687Q	1 L	■		■		
BE04-687F/U1	500 mL	■		■		■

Ordering Information – Dulbecco's Modified Eagle Medium:F12 (DMEM:F12)

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-719F	BE12-719F	Dulbecco's Modified Eagle Medium:F12 (DMEM:F12)	1:1 mixture with 3.151 g/L glucose, L-Glutamine and 15 mM HEPES	2°C to 8°C	500 mL
12-719Q	12-719Q	Dulbecco's Modified Eagle Medium:F12 (DMEM:F12)	1:1 mixture with 3.151 g/L glucose, L-Glutamine and 15 mM HEPES	2°C to 8°C	1 L
BE04-687F/U1	BE04-687F/U1	Dulbecco's Modified Eagle Medium:F12 (DMEM:F12)	1:1 mixture with 3.151 g/L glucose, with UltraGlutamine™ I, without HEPES	2°C to 8°C	500 mL
BE04-687Q	BE04-687Q	Dulbecco's Modified Eagle Medium:F12 (DMEM:F12)	1:1 mixture with 3.151 g/L glucose, without L-Glutamine or HEPES	2°C to 8°C	1 L

Glasgow Minimum Essential Medium (GMEM)

Designed to support BHK-21 cells.

2°C to 8°C

Ordering Information – Glasgow Minimum Essential Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-739F	BE12-739F	Glasgow Minimum Essential Medium	With L-Glutamine	2°C to 8°C	500 mL

Grace's Insect Medium

Classical insect cell media.

2°C to 8°C

Ordering Information – Grace's Insect Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
04-457F	04-457F	Grace's Insect Medium	Without FBS, yeastolate, lactalbumin hydrolysate or gentamicin	2°C to 8°C	500 mL
04-649F	04-649F	Grace's Insect Medium	Without FBS, with yeastolate, lactalbumin hydrolysate, and gentamicin	2°C to 8°C	500 mL

Related Products	Page
Insect-XPRESS™ Protein-free Insect Cell Medium	140
Schneider's Drosophila Medium	118
TC 100 Insect Medium	118

Ham's F10 Medium

2°C to 8°C

Developed for low density (clonal) growth of CHO cells.

Ordering Information – Ham's F10 Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
	BE02-014F	Ham's F10 Medium	With UltraGlutamine™ I, without thymidine	2°C to 8°C	500 mL
12-615F	BE12-615F	Ham's F12 Medium	With L-Glutamine	2°C to 8°C	500 mL
12-618F	BE12-618F	Ham's F10 Medium	With L-Glutamine	2°C to 8°C	500 mL

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Iscove's Modified Dulbecco's Medium (IMDM)

IMDM is suitable for fast growing cells. All formulas contain HEPES for added buffering.

2°C to 8°C

Cat. No.	Size	Without L-glutamine	With HEPES	Hybridoma Screened
12-722F	500 mL		■	
12-722Q	1 L		■	
12-726F	500 mL	■	■	
12-726Q	1 L	■	■	
12-915F	500 mL		■	■

Ordering Information – Iscove's Modified Dulbecco's Medium (IMDM)

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-722F	BE12-722F	Iscove's Modified Dulbecco's Medium (IMDM)	With L-Glutamine and 25 mM HEPES	2°C to 8°C	500 mL
12-722Q	12-722Q	Iscove's Modified Dulbecco's Medium (IMDM)	With L-Glutamine and 25 mM HEPES	2°C to 8°C	1 L
12-726F	BE12-726F	Iscove's Modified Dulbecco's Medium (IMDM)	Without L-Glutamine, with 25 mM HEPES	2°C to 8°C	500 mL
12-726Q	12-726Q	Iscove's Modified Dulbecco's Medium (IMDM)	Without L-Glutamine, with 25 mM HEPES	2°C to 8°C	1 L
12-915F	BE12-915F	Iscove's Modified Dulbecco's Medium (IMDM)	With L-Glutamine and 25 mM HEPES, hybridoma tested	2°C to 8°C	500 mL

L-15 (Leibovitz) Medium

Developed for fast growing tumor cells, this formula does not require a CO_2 enriched atmosphere. The bicarbonate-free medium is buffered with elevated levels of amino acids.

2°C to 8°C

Ordering Information – L-15 Leibovitz's Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-700Q	12-700Q	L-15 Leibovitz's Medium	Without L-Glutamine	2°C to 8°C	1 L
12-700F	BE12-700F	L-15 Leibovitz's Medium	Without L-Glutamine	2°C to 8°C	500 mL
12-669E	12-669E	L-15 Leibovitz's Modified Medium (2X)	(2X) except L-tyrosine (1X), without L-Glutamine or phenol red (virus plaquing medium)	2°C to 8°C	100 mL

McCoy's 5A Medium

Designed for human lymphocyte culture.

2°C to 8°C

Ordering Information – McCoy's 5A Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-168F	BE12-168F	McCoy's 5A Medium	With L-Glutamine and 25 mM HEPES	2°C to 8°C	500 mL
12-688F	BE12-688F	McCoy's 5A Medium	With L-Glutamine	2°C to 8°C	500 mL

Minimum Essential Medium – Eagle (MEM Eagle or E-MEM)

MEM Eagle is suitable for a diverse spectrum of mammalian cell types. It is available with either Hanks' or Earle's salts. MEM-Hanks' (12-127F or 12-137F) does not require a CO_2 enriched atmosphere. Joklik's modification is intended for suspension culture.

2°C to 8°C unless noted otherwise in the ordering information

Cat. No.	Size	With L-glutamine	With Sodium Pyruvate	Without Phenol Red	With HEPES	Powder	With UG I*	With HBSS	With EBSS	With NEAA	With Pen-strep-amph B	Without Na-bicarbonate	Without Calcium	With Nucleosides
12-169F (Alpha)	500 mL		■						■	■				
BE02-002F (Alpha)	500 mL		■				■		■	■				■
12-611F	500 mL	■							■					
12-611Q	1 L	■							■					
12-125F	500 mL								■					
12-125Q	1 L								■					
12-662F	500 mL		■						■	■				
12-662Q	1 L		■						■	■				
12-136F	500 mL				■				■					
12-136Q	1 L				■				■					
12-736E	100 mL	■			■				■	■	■			
12-736F	500 mL	■			■				■	■	■			
12-684F (10x)	500 mL											■		
12-668E (2x)	100 mL			■					■					
06-174G	450 mL	■							■	■			■	
12-127F	500 mL							■						
12-137F	500 mL				■			■						
04-719Q (Joklik's)	1 L	■							■				■	

* With UltraGlutamine I

Ordering information on the next page.

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Minimum Essential Medium – Eagle (MEM Eagle or E-MEM)

Continued

Ordering Information – Minimum Essential Medium – Alpha Eagle

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-169F	BE12-169F	Minimum Essential Medium - Alpha Eagle with Earle's BSS	Without L-Glutamine, deoxyribonucleosides and ribonucleosides	2°C to 8°C	500 mL
BE02-002F	BE02-002F	Minimum Essential Medium – Alpha Eagle	With UltraGlutamine™ I, deoxyribonucleoside and ribonucleosides	2°C to 8°C	500 mL
12-611F	BE12-611F	Minimum Essential Medium – Eagle with Earle's BSS	With L-Glutamine	2°C to 8°C	500 mL
12-611Q	12-611Q	Minimum Essential Medium – Eagle with Earle's BSS	With L-Glutamine	2°C to 8°C	1 L
12-125F	BE12-125F	Minimum Essential Medium – Eagle with Earle's BSS	Without L-Glutamine	15°C to 30°C	500 mL
12-125Q	12-125Q	Minimum Essential Medium – Eagle with Earle's BSS	Without L-Glutamine	15°C to 30°C	1 L
12-662F	BE12-662F	Minimum Essential Medium – Eagle with Earle's BSS	Without L-Glutamine, with non-essential amino acids and sodium pyruvate	2°C to 8°C	500 mL
12-662Q	12-662Q	Minimum Essential Medium – Eagle with Earle's BSS	Without L-Glutamine, with non-essential amino acids and sodium pyruvate	2°C to 8°C	1 L
12-136F	BE12-136F	Minimum Essential Medium – Eagle with Earle's BSS	Without L-Glutamine, with 25 mM HEPES	15°C to 30°C	500 mL
12-136Q	BE12-136Q	Minimum Essential Medium – Eagle with Earle's BSS	Without L-Glutamine, with 25 mM HEPES	15°C to 30°C	1 L
12-736E	12-736E	Minimum Essential Medium – Eagle with Earle's BSS	Cell culture maintenance medium 12-136 with non-essential amino acids, L-Glutamine, 25 mM HEPES, 10 µg/mL gentamicin, 50 units/mL penicillin, 50 µg/mL streptomycin, 2.5 µg/mL amphotericin B, and 2.0% heat-inactivated FBS	2°C to 8°C	100 mL
12-736F	12-736F	Minimum Essential Medium – Eagle with Earle's BSS	Cell culture maintenance medium 12-136 with non-essential amino acids, L-Glutamine, 25 mM HEPES, 10 µg/mL gentamicin, 50 units/mL penicillin, 50 µg/mL streptomycin, 2.5 µg/mL amphotericin B, and 2.0% heat-inactivated FBS	2°C to 8°C	500 mL
12-684F	BE12-684F	Minimum Essential Medium – Eagle with Earle's BSS (10X)	Without L-Glutamine or $NaHCO_3$	15°C to 30°C	500 mL
12-668E	12-668E	Minimum Essential Medium – Eagle with Earle's BSS (2X)	Without L-Glutamine or phenol red (virus plaquing medium)	15°C to 30°C	100 mL
BE12-668F	BE12-668F	Minimum Essential Medium – Eagle with Earle's BSS (2X)	Without L-Glutamine or phenol red (virus plaquing medium)	15°C to 30°C	500 mL
06-174G	BE06-174G	Minimum Essential Medium – Eagle with Earle's BSS	With non-essential amino acids, and L-Glutamine, without calcium	2°C to 8°C	450 mL
12-127F	BE12-127F	Minimum Essential Medium – Eagle with Hank's BSS	Without L-Glutamine	15°C to 30°C	500 mL
12-137F	12-137F	Minimum Essential Medium – Eagle with Hank's BSS	Without L-Glutamine, with 25 mM HEPES	15°C to 30°C	500 mL
04-719Q	04-719Q	Minimum Essential Medium – Eagle Joklik's Formulation	For suspension cultures, with L-Glutamine, without calcium	2°C to 8°C	1 L

Medium 199

Medium 199 was originally formulated for chick embryo fibroblast culture. These four formulations require a CO_2 enriched atmosphere.

2°C to 8°C

Cat. No.	Size	With L-glutamine	With HEPES	With HBSS	With EBSS	With 1.4 g/L Na-bicarbonate	With 2.2 g/L Na-bicarbonate
12-117F	500 mL	■	■		■		■
12-117Q	1 L	■	■		■		■
12-118F	500 mL	■	■	■		■	
12-119F	500 mL	■			■		■
12-109F	500 mL	■		■		■	

Ordering Information – Medium 199

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-109F	BE12-109F	Medium 199	With Hank's BSS, L-Glutamine, and 1.4 g/L $NaHCO_3$	2°C to 8°C	500 mL
12-117F	BE12-117F	Medium 199	With Earle's BSS, L-Glutamine, 25 mM HEPES, and 2.2 g/L $NaHCO_3$	2°C to 8°C	500 mL
12-117Q	12-117Q	Medium 199	With Earle's BSS, L-Glutamine, 25 mM HEPES, and 2.2 g/L $NaHCO_3$	2°C to 8°C	1 L
12-118F	BE12-118F	Medium 199	With Hank's BSS, L-Glutamine, 25 mM HEPES, and 1.4 g/L $NaHCO_3$	2°C to 8°C	500 mL
12-119F	BE12-119F	Medium 199	With Earle's BSS, L-Glutamine, and 2.2 g/L $NaHCO_3$	2°C to 8°C	500 mL

NCTC-109 Medium

A complex formula used to supplement hybridoma medium.

2°C to 8°C

Ordering Information – NCTC-109 Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-923E	12-923E	NCTC-109 Medium	With Earle's BSS and L-Glutamine, hybridoma screened	2°C to 8°C	100 mL

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RPMI 1640 Medium

RPMI is a general purpose media with a broad range of applications for mammalian cells, especially hematopoietic cells. RPMI with MOPS (04-525F) can be used with certain mycological assays.

2°C to 8°C unless otherwise noted in the ordering information

Cat. No.	Size	With L-glutamine	Without Phenol Red	With HEPES	Powder	With Ultra-Glutamine I	With Penicillin – Streptomycin	Without Na-bicarbonate	With MOPS Buffer	Without D-glucose
12-702F	500 mL	■								
12-702Q	1 L	■								
BE12-702F/U1	500 mL					■				
12-167F	500 mL									
12-167Q	1 L									
12-115F/U1	500 mL			■		■				
12-115F	500 mL	■		■						
12-115Q	1 L	■		■						
04-525F	500 mL	■						■	■	
BE04-558F	500 mL			■						
12-918F	500 mL		■							
09-774E	100 mL	■		■			■			
09-774F	500 mL	■		■			■			
BE12-752F	500 mL	■								■

Ordering Information – RPMI 1640 Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
	BE12-702F/U1	RPMI 1640 Medium	With UltraGlutamine™ I	15°C to 30°C	500 mL
	BE12-115F/U1	RPMI 1640 Medium	With UltraGlutamine™ I and 25 mM HEPES	15°C to 30°C	500 mL
04-525F	04-525F	RPMI 1640 Medium	For certain mycological assays. With L-Glutamine and 165 mM MOPS, without sodium bicarbonate	2°C to 8°C	500 mL
	BE04-558F	RPMI 1640 Medium	Without L-Glutamine, with 25mM HEPES	15°C to 30°C	500mL
09-774E	09-774E	RPMI 1640 Medium	With L-Glutamine, 25 mM HEPES, 100 units/mL penicillin, and 50 µg/mL streptomycin	2°C to 8°C	100 mL
09-774F	09-774F	RPMI 1640 Medium	With L-Glutamine, 25 mM HEPES, 100 units/mL penicillin, and 50 µg/mL streptomycin	2°C to 8°C	500 mL
12-115F	BE12-115F	RPMI 1640 Medium	With L-Glutamine and 25mM HEPES	2°C to 8°C	500 mL
12-115Q	12-115Q	RPMI 1640 Medium	With L-Glutamine and 25mM HEPES	2°C to 8°C	1 L
12-167F	BE12-167F	RPMI 1640 Medium	Without L-Glutamine	15°C to 30°C	500 mL
12-167Q	12-167Q	RPMI 1640 Medium	Without L-Glutamine	15°C to 30°C	1 L
12-702F	BE12-702F	RPMI 1640 Medium	With L-Glutamine	2°C to 8°C	500 mL
12-702Q	12-702Q	RPMI 1640 Medium	With L-Glutamine	2°C to 8°C	1 L
12-918F	BE12-918F	RPMI 1640 Medium	Without L-Glutamine or phenol red	15°C to 30°C	500 mL
BE12-752F	BE12-752F	RPMI 1640 Medium	With L-Glutamine, without D-glucose	2°C to 8°C	500 mL

Insect Media

2°C to 8°C

Ordering Information – Schneider's Drosophila Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
04-351Q	04-351Q	Schneider's Drosophila Medium	Modified, with L-Glutamine	2°C to 8°C	1 L

Ordering Information – TC 100 Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
	BE02-011F	TC 100 Insect Medium	With L-Glutamine	2°C to 8°C	500 mL

Related Products	Page
Grace's Insect Medium	112
Insect-XPRESS™ Protein-free Insect Cell Medium	140

William's Medium E

2°C to 8°C

Ordering Information – William's Medium E

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-019F	BE02-019F	William's Medium E	Without L-Glutamine or phenol red	2°C to 8°C	500 mL
	BE12-761F	William's Medium E	Without L-Glutamine	2°C to 8°C	500 mL

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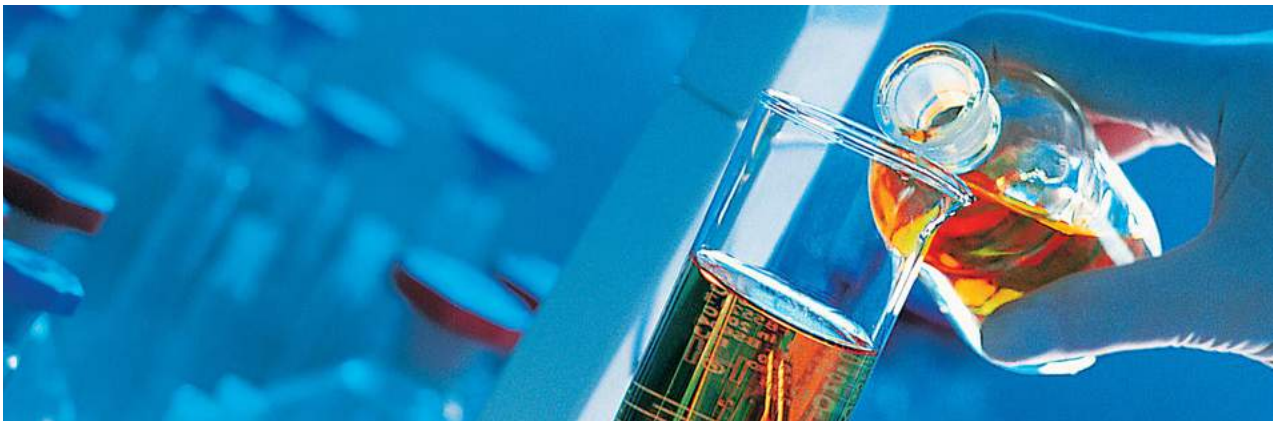
BioWhittaker™ Specialty Media



BioWhittaker™ Specialty Media

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TheraPEAK™ MSCGM™ CD Serum-free Mesenchymal Stem Cell Growth Medium – Chemically Defined	121
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Introduction

Serum-free media and reagents have a wide range of applications, including production of monoclonal antibodies, viral antigens, and recombinant proteins using a variety of mammalian and invertebrate cell lines. There are numerous advantages associated with the use of serum-free media formulations.

Benefits

- Increased definition
- Increased lot-to-lot consistency
- Simplified purification and downstream processing
- Better control over the physiological condition of cultures
- Ability to optimize formulations for specific cell types

Serum-free media formulations must satisfy a number of nutritional and physical requirements of cells that are normally addressed by the presence of serum. Serum proteins, such as albumin, fibronectin, and fetuin serve a variety of functions that include adsorbing toxic compounds, providing protection against shear forces in bioreactors, creating a matrix for cellular attachment to surfaces, and acting as a carrier for lipids and other growth factors.

For adaptation of cell cultures to serum-free media, see page 443.

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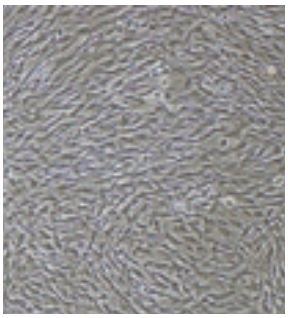
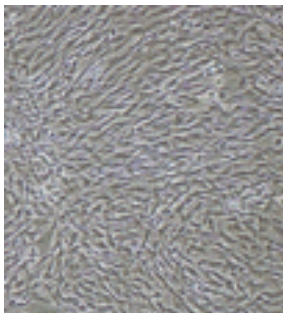
TheraPEAK™ MSCGM™ CD Serum-free Mesenchymal Stem Cell Growth Medium – Chemically Defined

TheraPEAK™ MSCGM™ CD Serum-free Mesenchymal Stem Cell Growth Medium – Chemically Defined (with L-glutamine, without phenol red and antibiotics).



Poietics™ hMSC grown in MSCGM™ CD Medium



Poietics™ hMSC grown in DMEM with 10% FBS

Benefits

- Supports human mesenchymal stem cell growth (multi-potent adult stem cells)
- Differentiation into osteoblasts, chondrocytes and adipocytes
- Chemically defined and serum-free

Basal media: 2°C to 8°C
SingleQuots™ Supplement: -10°C to -20°C

Ordering Information – TheraPEAK™ MSCGM™ CD Serum-free Mesenchymal Stem Cell Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
190620	190620	MSCBM™ CD Mesenchymal Stem Cell Basal Medium – Chemically Defined		2°C to 8°C	500 mL
192125	192125	MSCGM™ CD Mesenchymal Stem Cell Growth Medium SingleQuots™ Supplements and Growth Factors		-10°C to -20°C	Kit
190632	190632	MSCGM™ CD Mesenchymal Stem Cell Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Basal medium: 2°C to 8°C, Supplements: -10°C to -20°C	Kit

Related Products	Page
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130
hMSC – Human Mesenchymal Stem Cells	29

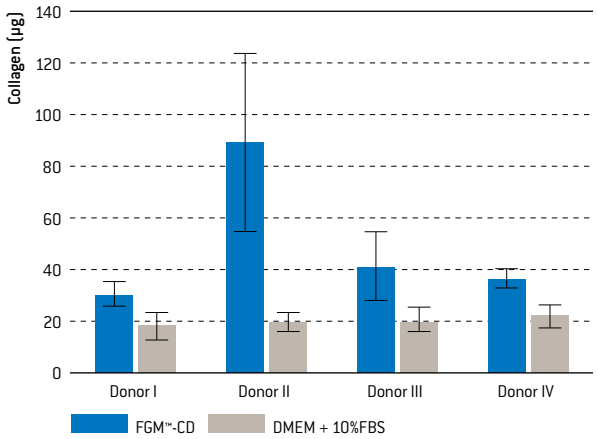
TheraPEAK™ FGM™ CD Serum-free Fibroblast Growth Medium – Chemically Defined

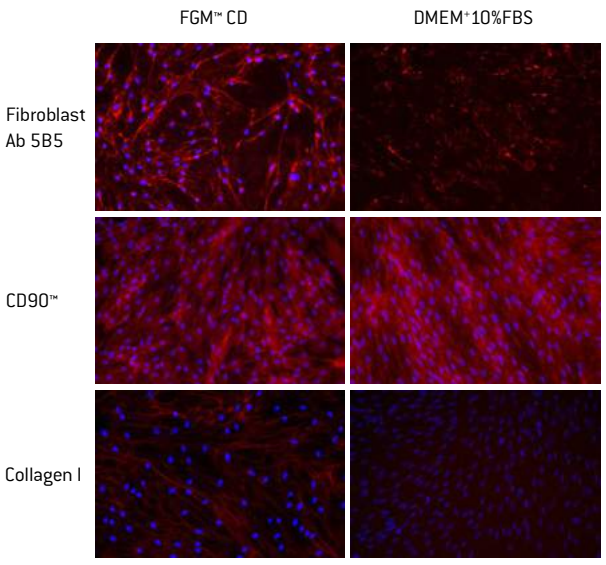
Benefits

- Serum-free and chemically defined medium for expansion
  - Adult – normal primary human fibroblasts
  - Neonatal – normal primary human fibroblasts
- Maintain the fibroblasts characteristics
- Promote collagen production
- TheraPEAK™ brand helps in transitioning from Research to Therapeutic applications

Basal media: 2°C to 8°C
SingleQuots™ Supplements: -10°C to -20°C

Collagen Production by NHDF-Ad grown in FGM™ CD





Ordering Information – TheraPEAK™ FGM™ CD Serum-free Fibroblast Growth Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
199019	199019	TheraPEAK™ FBM™ CD Fibroblast Basal Medium – Chemically-Defined		2°C to 8°C	500 mL
199020	199020	TheraPEAK™ FGM™ CD Fibroblast Growth Medium SingleQuots™ Supplements and Growth Factors		-10°C to -20°C	Kit
199041	199041	TheraPEAK™ FGM™ CD Fibroblast Growth Medium BulletKit™	Includes basal medium and SingleQuots™ Kit	Basal medium: 2°C to 8°C, Supplements: -10°C to -20°C	Kit

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UltraCULTURE™ Serum-free Medium – Chemically Defined

General Purpose Serum-free Medium

UltraCULTURE™ Serum-free Medium - Chemically Defined is a complete, all-purpose medium designed for the cultivation of a wide variety of adherent and non-adherent mammalian cell types. UltraCULTURE™ Medium can be used to support fusion of cells during hybridoma formation, growth of monocyte, macrophage, epithelial, and fibroblastic cell lines, and generation of virus particles for vaccine production. The medium is supplemented with recombinant human insulin, bovine transferrin, and a purified mixture of bovine serum proteins, including albumin. The total protein concentration of UltraCULTURE™ Medium is approximately 3 mg/mL.

UltraCULTURE™ Medium can be supplemented with Cryoprotective Medium (Cat. No. 12-132A) to cryopreserve cells in a serum-free environment. UltraCULTURE™ Medium does not contain L-glutamine; please add 5 mL of 200 mM L-glutamine solution (Cat. No. 17-605 or 17-905) prior to use.

The formulation for UltraCULTURE™ Medium has been submitted to the FDA as a Master File. Permission to cross-reference the Master File may be obtained by contacting the Regulatory Affairs Department.

Turbidity may develop in UltraCULTURE™ Medium; experiments have determined that the turbidity will not alter the performance of the product

Applications

- Cultivation of adherent and non-adherent mammalian cells
- Generation of viral particles for vaccine production

2°C to 8°C

Partial List of Cell Cultures Cultivated with UltraCULTURE™ Serum-free Medium - Chemically Defined

Cell Line	Source	Cell Type
HEL, N-10	Human	Fetal lung diploid fibroblast
HeLa	Human	Uterine cancer
HuL-1,2	Human	Liver (normal)
HuK-1	Human	Kidney (normal)
HuS-1AT	Human	Skin
HEC	Human	Embryonic cancer
HL-60	Human	Acute promyelocytic leukemia
Raji	Human	Burkitt's lymphoma
EB-3	Human	Burkitt's lymphoma
K-562	Human	Chronic myelocytic leukemia
HNK	Human	Neonatal kidney (primary)
HTC29	Human	Colon cancer
TT	Human	Medullary thyroid tumor
MB231	Human	Breast carcinoma
U138	Human	Glioma
FM3A	Mouse	Breast cancer
NS-1	Mouse	Myeloma
L	Mouse	Subcutaneous
P388D1	Mouse	Macrophage-like
P815	Mouse	Mast cell tumor
T3	Mouse	Pituitary
B82	Mouse	L cells – connective tissue
RPL-1	Rat	Peritoneum
RSP-2	Rat	Spleen
RLG-1	Rat	Lung
Lym-1	Rat	Lymph node
RCR-1	Rat	Brain
235-1, MMQ	Rat	Pituitary
GC, GH3	Rat	Pituitary
CA77	Rat	Medulary thyroid tumor
Rat-1	Rat	Fibroblast
JTC-12	Monkey	Kidney
COS1, COS7	African green monkey	SV40 transformed kidney

Ordering Information – UltraCULTURE™ Serum-free Medium - Chemically Defined, general purpose

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-725F	BE12-725F	UltraCULTURE™ Serum-free Medium - Chemically Defined	Without L-Glutamine	2°C to 8°C	500 mL

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130

PC-1™ Serum-free Medium – Chemically Defined

General Purpose Serum-free Medium

PC-1™ Serum-free Medium – Chemically Defined is a low-protein medium intended for the culture of primary cells and anchorage-dependent cell lines. PC-1™ Medium contains a complete HEPES buffering system with known amounts of insulin, transferrin, fatty acids, and proprietary proteins assembled under strict quality control procedures. PC-1™ Medium is intended for a variety of research and industrial applications and is formulated using defined components for optimal cell growth, while maintaining the lowest possible protein content.

Applications

– Cultivation of primary and anchorage-dependent cells

2°C to 8°C

Partial List of Cell Cultures Cultivated with PC-1™ Serum-free Medium – Chemically Defined

Cell Line	Source	Cell Type
HeLa	Human	Epithelial carcinoma, cervix
HTB-72	Human	Malignant melanoma, epithelial-like
HTB-4	Human	Bladder tumor
WI-38	Human	Lung, diploid
MRC-5	Human, male	Embryonal lung, fibroblast-like
BHK-21	Syrian hamster	Kidney, fibroblast-like
CHO-K1	Chinese hamster	Ovary, epithelial-like
NRK	Rat	Normal kidney, epithelial/fibroblast-like
C6	Rat	Glioma, primary
T9	Rat	Glioma
ARL6T	Rat	Normal liver
3T3	Mouse	Embryonic, fibroblast-like
STO	Mouse	Transformed fibroblast
VERO	African green monkey	Fibroblast
MDCK	Dog	Madin Darby canine kidney, epithelial-like
SIRC	Rabbit	Cornea

Other Cell Types/Sources

Source	Cell Type
Human:	Neuroblastoma, foreskin fibroblast, bladder carcinoma, renal papillary collecting tubule (primary), colon epithelium (primary), colon carcinoma (primary)
Rat:	Dermal fibroblast (primary), mammary carcinoma (primary), neonatal normal cardiac muscle (primary), thyroid epithelium (primary), astrocytes (primary)
Baboon:	(Paprocynocephalus) spinal ganglia
Swine:	Testes cell
Bovine:	Kidney

Ordering Information – PC-1™ Serum-free Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
77232	77232	PC-1™ Serum-free Medium – Chemically Defined	Complete medium system, including frozen supplement, without L-Glutamine. For primary adherent cells.	Medium: 2°C to 8°C, Supplement: -10°C to -20°C	2 × 500 mL

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130

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UltraMEM™ Reduced Serum Medium – Chemically Defined

General Purpose Serum-free Media

UltraMEM™ Reduced Serum Medium – Chemically Defined is designed to support growth and maintenance of several anchorage-dependent cell types under reduced serum concentrations (see table). When supplemented with 2–4% serum, UltraMEM™ Medium growth performance is comparable and, in some instances, exceeds that of standard media supplemented with 10% fetal bovine serum. Weaning is not necessary for most applications. Further reduction in serum concentration (<2%) can be achieved over several passages. In addition, confluent cultures can be maintained with minimal amounts of serum (≤1%) or no serum at all. Growth performance in UltraMEM™ Medium can be further increased by the addition of insulin, transferrin, and selenium to the basal medium UltraMEM™ Reduced Serum Medium, offered as a complete low protein medium supplemented with ITES. Recombinant human insulin and transferrin are the only protein components of the complete formulation and are present at a total concentration of 20 µg/mL.

Applications

– Growth and maintenance of anchorage-dependent cell types

2°C to 8°C

Partial List of Cell Cultures Cultivated with UltraMEM™ Reduced Serum Medium – Chemically Defined

Cell Type	Recommended % Serum
Diploid	
CEF, chicken fibroblast	2% FBS
WI-38, human fibroblast	3% FBS
RHMK, monkey kidney	2–4% FBS
MRC-5, human fibroblast	3% FBS
RK, rabbit kidney	3% FBS
BGMK, monkey kidney	2–3% FBS
HEL, human fibroblast	2–3% FBS
CMK, monkey kidney	3–4% FBS
Heteroploid	
VERO, monkey kidney	3% FBS
CRFK, cat kidney	2–3% FBS
L929, mouse fibroblast	4% FBS
MDCK, dog kidney	2–3% FBS
McCOY, mouse fibroblast	4% FBS
3T3, mouse fibroblast	3–4% FBS
CHO-K1, hamster ovary	3% FBS
PK-15, pig kidney	3–4% FBS
MK2, monkey kidney	3–4% FBS

Ordering Information – UltraMEM™ Reduced Serum Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-743F	BE12-743F	UltraMEM™ Reduced Serum Medium – Chemically Defined	Low protein medium with ITES and L-Glutamine	2°C to 8°C	500 mL

X-VIVO™ Serum-free Hematopoietic Cell Media – Chemically Defined

Serum-free Media for Hematopoietic Cells

X-VIVO™ Serum-free Hematopoietic Cell Media – Chemically Defined media provides a nutritionally complete and balanced environments for a variety of cells including lymphokine activated killer (LAK) cells, peripheral blood lymphocytes (PBL), and tumor infiltrating lymphocytes (TIL). These media do not contain any exogenous growth factors, artificial stimulators of cellular proliferation, or undefined supplements. They are devoid of any protein kinase C stimulators and are suitable for the investigation of second messenger systems in the activation of human and murine lymphocytes. The formulations are complete and contain pharmaceutical grade human albumin, recombinant human insulin, and pasteurized human transferrin.
All X-VIVO™ Media products are listed with the FDA in a Drug Master File. Permission to cross-reference the Master File may be obtained by contacting the Regulatory Affairs Department.

X-VIVO™ 10 Serum-free Hematopoietic Cell Media – Chemically Defined

The X-VIVO™ 10 Media formulations are designed to support the generation of LAK cells in a serum-free environment. The original protocols involved the incubation of patient or normal donor peripheral blood lymphocytes (PBL) at $1.0–3.0 \times 10^6$ cells/mL for a period of 3 days in the presence of 1,000 Cetus units of rIL-2/mL. Optimal LAK cell generation is achieved when peripheral blood lymphocytes are incubated for 3–10 days at a density of $1.0–6.0 \times 10^6$ cells/mL in the presence of 100–1,000 Cetus units of rIL-2. X-VIVO™ 10 Media is available as a 1X liquid in two convenient formulations.

X-VIVO™ 15 Serum-free Hematopoietic Cell Media – Chemically Defined

X-VIVO™ 15 Media are similar in composition to X-VIVO™ 10 Media and have been optimized for the proliferation of tumor infiltrating lymphocytes (TIL) under serum-free conditions. X-VIVO™ 15 Media supports the proliferation of purified $CD3^+$ cells isolated from peripheral blood and human tumors. X-VIVO™ 15 Media can also be used to support the growth of human monocytes, macrophage cells and cell lines, PBL, granulocytes, and natural killer (NK) cells. In addition, X-VIVO™ 15 Media provide a serum-free environment for the expansion of HUT-78 and related human lymphocytic cell lines.

X-VIVO™ 20 Serum-free Hematopoietic Cell Medium – Chemically Defined

X-VIVO™ 20 Medium is optimized to support the generation of lymphokine activated killer (LAK) cells from monocyte-depleted peripheral blood lymphocytes (PBL) at high density. Initial cell densities between $2.0–3.0 \times 10^7$ cells/ml can be used to successfully generate LAK cells. X-VIVO™ 20 Medium may also be used as a growth medium for PBL and tumor infiltrating lymphocytes (TIL).

Applications

- Proliferation of peripheral blood lymphocytes
- Proliferation of tumor infiltrating lymphocytes
- Cryopreservation and transplantation of organs
- Cultivation of human monocytes and macrophages
- Cultivation of stem cells
- Cultivation of dendritic cells

2°C to 8°C

www.lonza.com/xvivo

Ordering Information – X-VIVO™ Serum-free Hematopoietic Cell Media – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
04-743Q	BE04-743Q	X-VIVO™ 10 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine, without gentamicin or phenol red	2°C to 8°C	1 L
04-380Q	BE04-380Q	X-VIVO™ 10 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine, gentamicin, and phenol red	2°C to 8°C	1 L
	BE02-060F	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine, gentamicin, and phenol red	2°C to 8°C	500 mL
04-418Q	BE02-060Q	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine, gentamicin, and phenol red	2°C to 8°C	1 L
04-744Q	BE02-061Q	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine, without gentamicin or phenol red	2°C to 8°C	1 L
04-448Q	BE04-448Q	X-VIVO™ 20 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine, gentamicin and phenol red	2°C to 8°C	1 L

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X-VIVO™ Serum-free Hematopoietic Cell Media with Recombinant Transferrin – Chemically Defined

Ordering Information – X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-053Q	BE02-053Q	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine, gentamicin, recombinant transferrin, and phenol red	2°C to 8°C	1 L
BE02-054Q	BE02-054Q	X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined	With L-Glutamine and recombinant transferrin, without gentamicin or phenol red	2°C to 8°C	1 L
BE02-055Q	BE02-055Q	X-VIVO™ 10 Serum-free Hematopoietic Cell Medium – Chemically Defined	With recombinant transferrin, without gentamicin or phenol red	2°C to 8°C	1 L

UltraCHO™ Serum-free CHO Cell Medium

CHO Expression Media

UltraCHO™ Serum-free Cell Medium is optimized to support the growth of transfected and non-transfected Chinese Hamster Ovary (CHO) cells and expression of recombinant proteins. UltraCHO™ Media are composed of a modified DMEM:F-12 base and the formulation is supplemented with insulin, transferrin, and proprietary purified proteins. UltraCHO™ Medium contains less than 300 µg/mL protein.

UltraCHO™ Medium is manufactured under current ISO:9001 and the formulation has been submitted to the FDA as a Drug Master File. Permission to cross-reference the file may be obtained from the Regulatory Affairs Department.

Applications
- Growth of CHO cells
- Recombinant protein production

Partial list of cell types cultivated with UltraCHO™ Medium
- Transfected and non-transfected CHO cell lines
- HeLa cells (suspension or attached)
- Human leukemia cell lines

2°C to 8°C

Ordering Information – UltraCHO™ Serum-free CHO Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-724Q	12-724Q	UltraCHO™ Serum-free CHO Medium	With L-Glutamine	2°C to 8°C	1 L

ProCHO™ Protein-free CHO Media

Non-animal Origin CHO Expression Media

ProCHO™ Protein-free CHO Media were developed specifically to facilitate the production and downstream processing of recombinant proteins expressed in CHO cells. These protein-free formulations support high-density cultures without the need for animal derived components. Very low levels of recombinant insulin facilitate both downstream purification and regulatory compliance. The following media systems are available:

- ProCHO™ 4 Medium – For concurrent transition of adherent CHO cells to serum-free and suspension culture; supports faster doubling times
- ProCHO™ 5 Medium – For CHO cells already growing in suspension; supports increased protein production
- ProCHO™ AT Medium – For adherent culture of CHO cells

Ordering Information – ProCHO™ Protein-free CHO Cell Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
04-919Q	04-919Q	ProCHO™ 4 Protein-free CHO Medium	With 0.1% Pluronic® F-68, without L-Glutamine, phenol red, hypoxanthine or thymidine	2°C to 8°C	1 L
12-029Q	BE12-029Q	ProCHO™ 4 Protein-free CHO Medium	With 0.1% Pluronic® F-68 and phenol red, without L-Glutamine, hypoxanthine or thymidine	2°C to 8°C	1 L
	BE02-041Q	ProCHO™ 5 Protein-free CHO Medium	With 0.1% Pluronic® F-68, without L-Glutamine, phenol red, hypoxanthine, thymidine or glucose	2°C to 8°C	1 L
12-766Q	BE12-766Q	ProCHO™ 5 Protein-free CHO Medium	With 0.1% Pluronic® F-68, without L-Glutamine, phenol red, hypoxanthine or thymidine	2°C to 8°C	1 L
BE02-016Q	BE02-016Q	ProCHO™ AT Serum-free Medium	With L-Glutamine, without hypoxanthine or thymidine	2°C to 8°C	1 L

Related Products	Page
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130
ProHT™ (100X) Supplement	146
Glucose Solution	146

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PowerCHO™ Serum-free CHO Media – Chemically Defined

Non-animal Origin CHO Expression Media

PowerCHO™ 1, 2, and 3 Chemically Defined, Serum-free CHO Media are the next generation in CHO media, optimized for both cell growth and protein production. PowerCHO™ Media are hydrolysate-free, serum-free, and non-animal origin media for supporting high-density CHO cells in suspension. For therapeutic bioprocessing applications, these protein-free formulations also facilitate both downstream purification and regulatory compliance.

Benefits

- Maximum culture performance through balanced formulation
- Maintain high viability (>90%) at high cell densities
- Confidence in performance lot-to-lot with chemically defined, serum-free media
- Easily scaleable to support high-density, large scale production volumes

Application

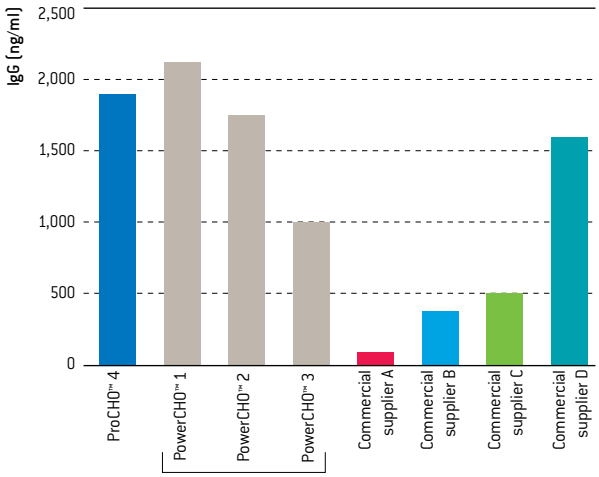
- Recombinant protein expression in CHO cells

2°C to 8°C

PowerCHO™-GS is designed for use with Lonza's proprietary GS Gene Expression System™.

www.lonza.com/gssystem

PowerCHO™ Media and ProCHO™ Media IgG Production



Ordering Information – PowerCHO™ Cell Media

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-770Q	12-770Q	PowerCHO™ 1 Serum-free Medium – Chemically Defined	With HEPES and Pluronic® F-68, without L-Glutamine, phenol red, hypoxanthine or thymidine	2°C to 8°C	1 L
BE02-042Q	BE02-042Q	PowerCHO™ 2 Serum-free CHO Medium – Chemically Defined	With HEPES and Pluronic® F-68, without L-Glutamine, phenol red, hypoxanthine, glucose or thymidine	2°C to 8°C	1 L
12-771Q	BE12-771Q	PowerCHO™ 2 Serum-free Medium – Chemically Defined	With HEPES and Pluronic® F-68, without L-Glutamine, phenol red, hypoxanthine or thymidine	2°C to 8°C	1 L
12-772Q	BE12-772Q	PowerCHO™ 3 Serum-free Medium – Chemically Defined	With HEPES and Pluronic® F-68, without L-Glutamine, phenol red, hypoxanthine or thymidine	2°C to 8°C	1 L
BE12-776Q	BE12-776Q	PowerCHO™ GS Serum-free CHO Medium – Chemically Defined	With HEPES and Pluronic® F-68, without L-Glutamine, insulin or phenol red	2°C to 8°C	1 L

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130
Glucose Solution	146

PowerCHO Advance™ – Chemically Defined

Non-animal Origin Medium

PowerCHO Advance™ is a chemically defined, non-animal origin media that allows for expansion of CHO cells under serum-free conditions. PowerCHO Advance™ has been shown to provide higher protein titer when compared with leading media formulations on the market. PowerCHO Advance™ also allows for easy scale up due to improved filterability.

Benefits

- Regulatory friendly
- Allows for easy scalability
- Easily paired with fed batch systems for scale-up studies
- Easy filterability

2°C to 8°C

Ordering Information – PowerCHO Advance™ – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-929Q	12-929Q	PowerCHO Advance™ – Chemically Defined	Non-Animal Origin	2°C to 8°C	1 L

ProFreeze™ CD (2X), NAO Freeze Medium – Chemically Defined

Non-animal Origin Freezing Medium

ProFreeze™ CD (2X), NAO Freeze Medium – Chemically Defined is universally suitable for cryopreserving many cell types in the absence of fetal bovine serum (FBS). However, it is used to greatest advantage with cells cultured in a serum-free and animal component-free environment. This protein-free freezing medium contains no animal derived components, insulin, or hydrolysate, and maintains high cell viability upon recovery from frozen storage.

ProFreeze™ Medium requires the addition of 15% reagent or spectrophotometric grade dimethylsulfoxide (DMSO) at time of use. One bottle will make 117.6 mL of complete 2X concentrated freezing medium after the addition of 17.6 mL DMSO. For best results, keep on ice during use.

2°C to 8°C

Ordering Information – ProFreeze™ CD (2X) Freeze Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-769E	12-769E	ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	Non-Animal Origin	2°C to 8°C	100 mL

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UltraMDCK™ Serum-free Renal Cell Medium – Chemically Defined

Renal Cell Expression Medium

UltraMDCK™ Serum-free Renal Cell Medium – Chemically Defined serum-free medium designed to support the growth of MADIN-DARBY Canine Kidney (MDCK) cells at low and high plating densities. UltraMDCK™ Medium contains low levels of recombinant human insulin and bovine transferrin, yielding a very low protein formulation. MDCK cells grown in UltraMDCK™ Medium are smaller and more densely packed than cells grown in the presence of serum, and cultures can stay confluent for at least two weeks without medium change.

Applications

– Growth of kidney cells including MDCK cells

2°C to 8°C

Ordering Information – UltraMDCK™ Serum-free Renal Cell Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-7490	BE12-7490	UltraMDCK™ Serum-free Renal Cell Medium – Chemically Defined	With L-Glutamine	2°C to 8°C	1 L

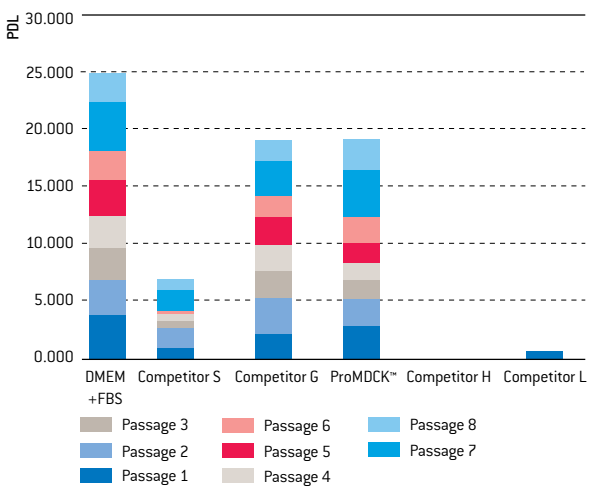
ProMDCK™ 2D Medium

Non-animal Origin

ProMDCK™ 2D is a non-animal-origin (NAO) serum-free medium that supports the growth of Madin-Darby Canine Kidney Cells (MDCK) in cell culture. ProMDCK™ 2D medium is optimized for expansion and virus infection of MDCK cells in planar culture (2D).

Benefits

– A completely defined, serum-free, non-animal origin medium
– Supports the proliferation of MDCK cells in planar culture
– Easy transition from 2D to 3D MDCK cultures
– Optimal cell proliferation compared to competing media products



2°C to 8°C

www.lonza.com/promdck

Ordering Information – ProMDCK™ 2D Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-9260	12-9260	ProMDCK™ 2D Medium	Seurm-free, Non-Animal Origin	2°C to 8°C	1 L

Pro293™ Serum-free Media – Chemically Defined

Non-animal Origin Renal Cell Expression Media

Pro293™ Serum-free Media – Chemically Defined were optimized to support high-density growth and recombinant protein production in 293 neonatal kidney cells. They are chemically defined to ease regulatory compliance and downstream protein purification. They contain very low levels of recombinant human insulin, and are free of animal-origin components.

- Pro293™s Medium – for 293 cells growing in suspension culture or to be suspension adapted
- Pro293™s Medium – for 293 cells growing in adherent culture

Applications

- Recombinant protein production in 293 cells

2°C to 8°C

Ordering Information – Pro293™ Serum-free Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-765Q	12-765Q	Pro293™s Serum-free Medium – Chemically Defined for 293 Cells in Suspension	With 0.1% Pluronic® F-68, without L-Glutamine or phenol red	2°C to 8°C	1 L
BE02-025Q	BE02-025Q	Pro293™s Serum-free Medium – Chemically Defined for 293 Cells in Suspension	With 0.1% Pluronic® F-68, without L-Glutamine, galactose or phenol red	2°C to 8°C	1 L
12-764Q	BE12-764Q	Pro293™a Serum-free Medium – Chemically Defined for 293 Adherent Cells	With 0.1% Pluronic® F-68, without L-Glutamine or phenol red	2°C to 8°C	1 L

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130

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ProVero™ 1 Serum-free Medium

Non-animal Origin Renal Cell Expression Medium

ProVero™ 1 Serum-free Medium is a protein-free medium designed to support the growth of MDCK and Vero cells. ProVero™ 1 Medium includes HEPES and sodium bicarbonate buffer. The absence of proteins and only very low levels of human recombinant insulin facilitate both downstream processing and regulatory compliance.

Some Vero cell strains require additional supplementation with 5.0 µg/L rhEGF for optimal Vero cell growth.

Applications

- Recombinant protein production
- Virus production

2°C to 8°C

Ordering Information – ProVero™ 1 Serum-free Medium

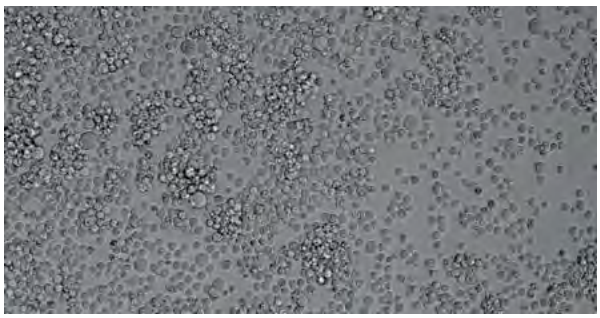
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-030Q	BE02-030Q	ProVero™ 1 Serum-free Medium	With L-Glutamine	2°C to 8°C	1 L

Related Products	Page
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130

ProPer™ 1 Serum-free Medium – Chemically Defined

Non-animal Origin Medium for Human Embryonic Retinoblast Cells

ProPer™ 1 Serum-free Medium – Chemically Defined is an animal origin component-free, chemically defined, serum-free medium for growth of human embryonic retinoblast cells (PER.C6® and related cell lines) in suspension. The medium contains a low amount of human recombinant insulin. HEPES as well as sodium bicarbonate are present in the formulation.



PER.C6® Cells growing in ProPer™ 1 Medium.

Applications

- Recombinant protein and virus production
- Growth of human embryonic retinoblast cells (PER.C6® and related cell lines) in suspension

2°C to 8°C

Ordering Information – ProPer™ 1 Serum-free Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-028Q	BE02-028Q	ProPer™ 1 Serum-free Medium – Chemically Defined	With 0.1% Pluronic® F-68, without L-Glutamine or phenol red	2°C to 8°C	1 L

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130

PERMEXCIS® Serum-free Virus Production Medium – Chemically Defined

Non-animal Origin Medium for Human Embryonic Retinoblast Cells

PER.C6® Technology is a human cell-based platform designed for the large-scale production of recombinant proteins, including antibodies, vaccines and gene therapy products, under serum-free culture conditions. Cell lines have been immortalized with specific adenovector genes, leading to high viable cell densities and high PCDs (picogram/cell/day), resulting in high yields.
PERMEXCIS® Virus Production Medium has been optimized for use with the PER.C6® Cell Line and is available to all PER.C6® Cell License Holders. PERMEXCIS® Medium is chemically defined, serum-free, low protein (<200 ng/mL), with Pluronic® F-68, and without L-glutamine or phenol red.

Applications
- Virus production
- Growth of human embryonic retinoblast cells (PER.C6® and related cell lines) in suspension

2°C to 8°C

Ordering Information – PERMEXCIS® Serum-free Virus Production Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-039Q	BE02-039Q	PERMEXCIS® Virus Production Medium – Chemically Defined	Without L-Glutamine, phenol red or antibiotics	2°C to 8°C	1 L

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130
UltraGlutamine™	147

Lymphochrome™ Serum-free Medium

Karyotyping Medium

Lymphochrome™ Serum-free Medium is a complete, ready-to-use, medium for the cultivation of lymphocytes from peripheral blood. Serum-free medium provides a high lot-to-lot consistency with high mitotic index and good chromosome pattern. For *in vitro* diagnostic use.

-10°C to -20°C

Ordering Information – Lymphochrome™ Serum-free Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
	BE02-015E	Lymphochrome™ Serum-free Medium	With L-Glutamine and phytohaemagglutinin	-10°C to -20°C	100 mL

CE marked according to IVD Directive 98/79/EC.

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

Amniochrome™ II Modified Medium

Cytogenetics Medium

Amniochrome™ II Modified Medium is for the culture of human amniotic fluid cells obtained from amniocentesis, a procedure extensively used for clinical prenatal diagnosis. The Amniochrome™ II Modified Medium is performance tested by a cytogenetic reference laboratory for the cultivation of amniotic fluid cells for cytogenetic analysis.

Complete medium for the primary culture of amniotic fluid and chorionic villi cells used in cytogenetic applications. The system includes an enriched basal medium and growth supplement. For diagnostic use.

Basal medium: 2°C to 8°C
Supplement: -10°C to -20°C

Ordering Information – Amniochrome™ II Modified Medium

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
	BE12-756EZM	Amniochrome™ II Modified Medium	Basal medium: 2°C to 8°C, Supplement: -10°C to -20°C	100 mL
	BE12-756FCM	Amniochrome™ II Modified Medium	Basal medium: 2°C to 8°C, Supplement: -10°C to -20°C	500 mL

CE marked according to IVD Directive 98/79/EC.

Amniochrome™ Plus Medium

Cytogenetics Medium

Complete, ready-to-use medium for the primary culture of amniotic fluid and chorionic villi cells used in cytogenetic applications. Quick attachment of the cells and high growth speed of cells are the main advantages of this new media formulation. For *in vitro* diagnostic use.

-10°C to -20°C

Ordering Information – Amniochrome™ Plus Medium

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
	BE02-026E	Amniochrome™ Plus Medium	-10°C to -20°C	100 mL
	BE02-026F	Amniochrome™ Plus Medium	-10°C to -20°C	500 mL

CE marked according to IVD Directive 98/79/EC.

Amniochrome™ Pro Medium

Cytogenetics Medium

Complete, ready-to-use medium for the primary culture of amniotic fluid and chorionic villi cells used in cytogenetic applications. Contains the necessary growth factors, L-glutamine, phenol red, sodium bicarbonate, and FBS. The complete formulation reduces handling steps and the possibility of contamination. For diagnostic use.

-10°C to -20°C

Ordering Information – Amniochrome™ Pro Medium

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
	BE02-035E	Amniochrome™ Pro Medium	-10°C to -20°C	100 mL
	BE02-035F	Amniochrome™ Pro Medium	-10°C to -20°C	500 mL

CE marked according to IVD Directive 98/79/EC.

HL-1™ Serum-free Medium – Chemically Defined

Chemically Defined Hybridoma Medium

HL-1™ Serum-free Medium – Chemically Defined is a culture medium containing less than 30 µg protein per mL. Components of HL-1™ Medium include HEPES buffer, insulin, transferrin, sodium selenite, ethanolamine, a variety of saturated and unsaturated fatty acids and proprietary stabilizing proteins. HL-1™ Medium contains no bovine serum albumin or other undefined protein mixtures. HL-1™ Medium supports the serum-free growth of various hybridomas, including those derived from P3X63Ag8.653 and Sp2/0-Ag14 myelomas, as well as other differentiated cells of lymphoid origin.

Applications

– Serum-free growth of hybridomas and differentiated cells of lymphoid origin

2°C to 8°C

HL-1™ FBS Substitute (100X) – Chemically Defined

HL-1™ Chemically Defined FBS Substitute (100X) is a chemically defined medium additive that can be used to replace serum or significantly reduce its concentration in a variety of basal media. It contains less than 30 µg protein per mL when diluted 1:100 in medium and it does not contain bovine serum albumin or other undefined protein ingredients.

15°C to 30°C

Ordering Information – HL-1™ Serum-free Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
77227	77227	HL-1™ Fetal Bovine Serum Substitute (100X) – Chemically Defined		15°C to 30°C	10 mL
77201	BE77201	HL-1™ Serum-free Medium for Hybridoma and Hematopoietic Cells – Chemically Defined	Without L-Glutamine	2°C to 8°C	2 × 500 mL

CE marked according to IVD Directive 98/79/EC.

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130

See next page for a partial list of cell cultures cultivated with HL-1™ Serum-free Medium-Chemically Defined.

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HL-1™ Serum-free Medium – Chemically Defined

Continued

Partial list of cell cultures cultivated with HL-1™ Serum-free Medium – Chemically Defined

Transformed and Established Cell Lines

Cell Line	Source	Cell Type
U937	Human	Macrophage
RaJi	Human	B lymphoblastic
MCF-7 (NIH)	Human	Breast carcinoma
MCF-7 (MCF)	Human	Breast carcinoma
NIH ZR-75	Human	Breast carcinoma
COLO 302 HSR	Human	Colon carcinoma
J82	Human	Bladder carcinoma
SW 1738	Human	Bladder carcinoma
SW780	Human	Bladder carcinoma
CCL 119	Human	Lymphoid
CCL 213	Human	Burkitt lymphoma
C91/PL	Human	T lymphoma
Undesignated	Human	Astrocytoma
Undesignated	Human	Hepatoma
MOLT-3	Human	Acute lymphoblastic leukemia
MOLT-4	Human	Acute lymphoblastic leukemia
NAMALWA	Human	Burkitt lymphoma
THP-1	Human	Monocytic leukemia
BB88	Mouse	Erythroid (leukemia)
P815	Mouse	Macrophage
P388D1	Mouse	Macrophage
WeHi3	Mouse	Monocyte
JLS-V5	Mouse	Spleen cell
70Z-3	Mouse	Pre-B lymphoma
70Z/3.12	Mouse	B lymphoma
S49 and variants	Mouse	T lymphoma
RAW309F1.1	Mouse	T lymphoma
WeHi7	Mouse	T lymphoma
I-10	Mouse	Leydig-tumor
EL4	Mouse	T lymphoma
RL1	Mouse	T lymphoma
BW5147.3	Mouse	T lymphoma
LBRM-33	Mouse	T lymphoma
Friend leukemia	Mouse	Leukemia
C57BL6	Mouse	Embryo (C57 × DBA)
L5178Y	Mouse	Lymphoma (DBA/2)
VERO	African green monkey	Fibroblast
MDCK	Dog	Madin Darby canine kidney
CHO K1	Hamster	Chinese hamster ovary (epithelial-like)
GCL2	Hamster/mouse	B lymphoma × Normal B

Hybridomas

Hybridoma	Source	Fusion Partner
8A1	Human	CLLC
undesignated	Human	WI-L2-729-HF2
undesignated	Human	LICR-LON-HMY2
HB44	Mouse	Sp2/0-Ag14
HB45	Mouse	Sp2/0-Ag14
HB56	Mouse	NS-1
HB59	Mouse	NS-1
HB60	Mouse	P3X63Ag 8.653
53-7.313	Mouse	NS-1
MI/9.3.4HL-2	Mouse	NS-1
MI/70.15.1	Mouse	NS-1
ARB	Mouse	Hybridoma
P3U	Mouse	P3X63Ag 8.653
BCS12	Mouse	P3X63Ag 8.653
BCS 2002	Mouse	P3X63Ag 8.653
undesignated	Mouse	NS-1
undesignated	Mouse	P3X63Ag 8.653
TIB 175	Rat/mouse	S194
TIB 104	Rat/mouse	NS-1
TIB 105	Rat/mouse	NS-1
TIB 109	Rat/mouse	NS-1
TIB 128	Rat/mouse	NS-1
TIB 166	Rat/mouse	NS-1
TIB 168	Rat/mouse	NS-1
RS	Rat/mouse	P3X63Ag 8.653

Primary Cells

Cell Type
Human peripheral blood mononuclear cells
Mink lymphocytes
Human fetal adrenal
Human blood monocytes
Human peripheral blood T lymphocytes

UltraDOMA™ Serum-free Hybridoma Medium – Chemically Defined

Hybridoma Medium

UltraDOMA™ Serum-free Hybridoma Medium is a formulation designed for the cultivation of murine, human, and chimeric hybridomas in batch culture and in hollow fiber bioreactors. UltraDOMA™ Medium is supplemented with recombinant human insulin, bovine transferrin and bovine albumin. The total protein concentration is 30 µg per mL. UltraDOMA™ Medium does not contain L-glutamine or human-derived proteins.

Cells that are adapted for growth in UltraDOMA™ Medium can be maintained in the medium indefinitely and can be cryopreserved in UltraDOMA™ Medium supplemented with Cryoprotective Freezing Medium (Cat. No. 12-132A).

Applications

- Hybridoma cell growth
- Monoclonal antibody production

2°C to 8°C

Partial List of Cell Types Cultivated with UltraDOMA™ Serum-free Hybridoma Medium

Cell Type
Murine hybridomas
NS-1 derived myelomas
SP-2 derived myelomas
Human hybridomas (with 0.5% FBS)

Ordering Information – UltraDOMA™ Serum-free Hybridoma Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-723B	12-723B	UltraDOMA™ Serum-free Hybridoma Medium	Without L-Glutamine	2°C to 8°C	500 mL (glass bottle)
	BE12-723F	UltraDOMA™ Serum-free Hybridoma Medium	Without L-Glutamine	2°C to 8°C	500 mL (plastic bottle)

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130
UltraGlutamine™	147

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UltraDOMA-PF™ Protein-free Hybridoma Medium – Chemically Defined

Non-animal Origin Hybridoma Media

UltraDOMA-PF™ Protein-free Hybridoma Medium is designed for use with hybridoma cell lines of murine, human, and chimeric origin. UltraDOMA-PF™ Medium is completely defined and does not contain peptides or tissue extracts. The use of UltraDOMA-PF™ Medium significantly simplifies downstream processing since all proteins present in a given cell culture supernatant are produced by the cells. UltraDOMA-PF™ Medium is designed for lab scale or industrial scale use. L-Glutamine and HEPES buffer are both included in the formulation.

Applications

– Hybridoma and myeloma growth
– Monoclonal antibody production

2°C to 8°C

Partial List of Cell Types Cultivated with UltraDOMA-PF™ Hybridoma Medium

Cell Type
Murine hybridomas
NS-1 derived myelomas
SP-2 derived myelomas
Human hybridomas (with 0.5% FBS)
Rat hybridomas
Some transfected Chinese Hamster Ovary (CHO) cell lines
Human lymphoid origin cells
Murine lymphoid origin cells

Ordering Information – UltraDOMA™ Protein-free Hybridoma Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-727F	12-727F	UltraDOMA™ Protein-free Hybridoma Medium	With L-Glutamine	2°C to 8°C	500 mL

ProDoma™ Serum-free Hybridoma Media

Non-animal Origin Hybridoma Media

ProDoma™ Serum-free Hybridoma Media is designed for cultivation of murine, human, and chimeric hybridomas. ProDoma™ Media are protein-free with a low amount of human recombinant insulin. All ProDoma™ Media include HEPES as well as sodium bicarbonate in the formulation.

Applications

– Hybridoma cell growth
– Monoclonal antibody production

2°C to 8°C

Ordering Information – ProDOMA™ Serum-free Hybridoma Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-029Q	BE02-029Q	ProDOMA™ 1 Serum-free Hybridoma Medium – Chemically Defined	With 0.1% Pluronic® F-68, without L-Glutamine or phenol red	2°C to 8°C	1 L
BE02-032Q	BE02-032Q	ProDOMA™ 3 Serum-free Hybridoma Medium	With 0.1% Pluronic® F-68, without L-Glutamine or phenol red	2°C to 8°C	1 L

Insect-XPRESS™ Protein-free Insect Cell Medium

Insect Cell Expression Medium

Insect-XPRESS™ Protein-free Insect Cell Medium is a formulation designed to support the growth of insect cell lines derived from *Spodoptera frugiperda* (Sf9 and Sf21). Cell densities in excess of 8.3 × 10^6 cells/mL can be achieved with suspension cultures of Sf9 cells using Insect-XPRESS™ Medium and an excess of oxygen. This formulation can also be used for stationary monolayer cultures and shake-flask cultures. Insect-XPRESS™ Medium contains L-glutamine and supports superior production of recombinant proteins when using the Baculovirus Expression Vector System (BEVS). For cryopreservation of insect cells, Insect-XPRESS™ Medium can be mixed 50:50 with Cryoprotective Freeze Medium (Cat. No. 12-132A).

2°C to 8°C

Ordering Information – Insect-XPRESS™ Protein-free Insect Cell Medium

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-730F	BE12-730F	Insect-XPRESS™ Protein-free Insect Cell Medium	With L-Glutamine	2°C to 8°C	500 mL
12-730Q	BE12-730Q	Insect-XPRESS™ Protein-free Insect Cell Medium	With L-Glutamine	2°C to 8°C	1 L

Related Products	Page
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130
Grace's Insect Medium	112
Schneider's Drosophila Medium	118

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ProNSO™ Protein-free Media – Chemically Defined

Non-animal Origin NSO Expression Media

The NSO cell line is widely used for recombinant mammalian protein expression. Some of the reasons to select this mouse myeloma platform are:

- Forms stably producing hybrid cells with high levels of protein production
- Lacks ability to secrete endogenous antibody or antibody fragments
- Resists aggregation clumping in suspension culture
- Lacks adverse protease activity on recombinant protein product (in many cases)

ProNSO™ 1 or 2 Protein-free Media – Chemically Defined Media, together with ProNSO™ Lipid CD Supplement, are designed to meet a growing demand for optimized NSO formulations.

- Two optimized formulas are available to cover the broadest range of NSO nutritional needs
- Further maximize protein production by titration with ProNSO™ Lipid CD Supplement
- Protein-free and chemically defined formulations
- Product purification is simplified
- Lot-to-lot consistency ensures dependable performance
- Non-animal origin components reduce regulatory burdens

Data in the figure to the right shows both ProNSO™ 1 and 2 CD Media yield superior protein production compared to competitive media.

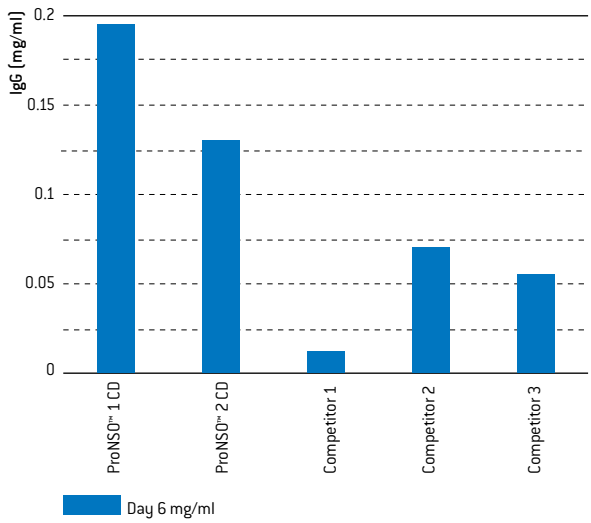
Applications

- High density culture of NSO cells

Basal media: 2°C to 8°C
Lipid supplement: -10°C to -20°C

ProNSO-2 available as a custom only. Contact your local sales representative for additional information.

Protein Production – ProNSO™ Media



ProNSO™ Media outperforms competitive media for recombinant IgG production in NSO cells. Duplicate 125 mL shaker flasks were seeded at a density of 200,000 NSO cells per mL in a 30 mL volume. Shake rate was 100 rpm. Cells were cultured in their respective test media for one passage prior to test initiation.

Ordering Information – ProNSO™ Protein-free Medium – Chemically Defined

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
12-773Q	12-773Q	ProNSO™ 1 Protein-free Medium – Chemically Defined	With HEPES and Pluronic®, without L-Glutamine, phenol red or cholesterol	2°C to 8°C	1 L
12-775J	12-775J	ProNSO™ Lipid Supplement – Chemically Defined	With cholesterol, suggested use is 5 mL/L	-10°C to -20°C	5 mL

Related Products	Page
L-Glutamine	146
ProFreeze™ CD (2X) Freeze Medium – Chemically Defined	130

Notes

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BioWhittaker™ Cell Culture Reagents

BioWhittaker™ Cell Culture Reagents

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Introduction

BioWhittaker™ Cell Culture Reagents include a number of different products, such as amino acids, antibiotics, and buffers, all of which are used routinely in research, and manufacturing applications involving cell culture.
These products are labeled for research use only and are manufactured under the same ISO:9001 conditions as our other cell culture products.

Chemicals we use to prepare cell culture reagents are purchased according to the raw material qualifications from approved suppliers. Each lot must meet established component specifications before it is released by Quality Assurance for use. We manufacture all liquid cell culture reagents using Water for Injection (WFI) quality water, which has been prepared by ultrafiltration, reverse osmosis, deionization, and distillation. Liquid products are sterile filtered through pharmaceutical-grade sterilizing filters.

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Earle's Balanced Salt Solution (BSS)

15°C to 30°C

Ordering Information – Earle's Buffered Saline Solution

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
	BE10-502F	Earle's Buffered Saline Solution	With phenol red	15°C to 30°C	500 mL
	BE02-027F	Earle's Buffered Saline Solution	With 20 mM HEPES, 1.8 g/L sodium bicarbonate and phenol red	15°C to 30°C	500 mL

Hank's Balanced Salt Solution (BSS)

15°C to 30°C

Cat. No.	Size	With Phenol Red	With Calcium and Magnesium
10-508F	500 mL	■	■
10-508Q	1 L	■	■
10-543F	500 mL	■	
10-543Q	1 L	■	
10-527F	500 mL		■
10-547F	500 mL		
04-315Q	1 L		

Ordering Information – Hank's Buffered Saline Solution

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
10-508F	BE10-508F	Hank's Buffered Saline Solution	With phenol red, calcium and magnesium	15°C to 30°C	500 mL
10-508Q	10-508Q	Hank's Buffered Saline Solution	With phenol red, calcium and magnesium	15°C to 30°C	1 L
10-543Q	10-543Q	Hank's Buffered Saline Solution	With phenol red, without calcium or magnesium	15°C to 30°C	1 L
10-543F	BE10-543F	Hank's Buffered Saline Solution	With phenol red, without calcium or magnesium	15°C to 30°C	500 mL
10-527F	BE10-527F	Hank's Buffered Saline Solution	Without phenol red, with calcium and magnesium	15°C to 30°C	500 mL
04-315Q	04-315Q	Hank's Buffered Saline Solution	Without phenol red, calcium or magnesium	15°C to 30°C	1 L
10-547F	BE10-547F	Hank's Buffered Saline Solution	Without phenol red, calcium or magnesium	15°C to 30°C	500 mL

Ordering Information – BioWhittaker™ Cell Culture Reagents

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-040E	BE02-040E	Glucose Solution	100 mg/mL	15°C to 30°C	100 mL
17-839Z	17-839Z	ITES (500X)	Supplement of insulin, transferrin, selenium and ethanolamine	-10°C to -20°C	5 mL
17-838Z	17-838Z	ITS (500X)	Supplement of insulin, transferrin and selenium	-10°C to -20°C	5 mL
17-905C	17-905C	L-Glutamine, 200 mM	Supplied at 29.3 mg/mL in 0.85% NaCl and hybridoma screened	-10°C to -20°C	50 mL
17-605C	17-605C	L-Glutamine, 200 mM	Supplied at 29.3 mg/mL in 0.85% NaCl	-10°C to -20°C	50 mL
17-605F	BE17-605F	L-Glutamine, 200 mM	Supplied at 29.3 mg/mL in 0.85% NaCl	-10°C to -20°C	500 mL
17-605E	BE17-605E	L-Glutamine, 200 mM	Supplied at 29.3 mg/mL in 0.85% NaCl	-10°C to -20°C	100 mL
17-829E	17-829E	Lymphocyte Separation Medium, 1.077	Density 1.077, for the isolation of human Lymphocytes	15°C to 30°C	100 mL
17-829F	17-829F	Lymphocyte Separation Medium, 1.077	Density 1.077, for the isolation of human Lymphocytes	15°C to 30°C	500 mL
13-607C	13-607C	MEM Eagle Vitamine Mixture (100X)		-20°C	50 mL
13-114E	BE13-114E	MEM Non-Essential Amino Acid Solution (100X)	Contains a 10 mM concentration of each non-essential amino acid	2°C to 8°C	100 mL
BE17-855E	BE17-855E	ProHT™ Supplement (100X)	Hypoxanthine, Thymidine supplement (100X) from non-animal origin, optimized for use with ProCHO™ Media	-10°C to -20°C	100 mL
17-613E	BE17-613E	Sodium Bicarbonate Solution, 7.5%	7.5% aqueous solution	15°C to 30°C	100 mL
13-115E	BE13-115E	Sodium Pyruvate Solution (100 mM)	11.1 g/L	2°C to 8°C	100 mL
17-942E	17-942E	Trypan Blue, 0.4% Solution	Prepared in 0.85% NaCl	15°C to 30°C	100 mL
17-160F	17-160F	Trypsin 1:250 (10X)	2.5% in modified Hanks' BSS without calcium or magnesium, manufactured with irradiated porcine trypsin, tested for porcine parvovirus and mycoplasma	-10°C to -20°C	500 mL
17-160E	BE17-160E	Trypsin 1:250 (10X)	2.5% in modified Hanks' BSS without calcium or magnesium, manufactured with irradiated porcine trypsin, tested for porcine parvovirus and mycoplasma	-10°C to -20°C	100 mL
BE02-007E	BE02-007E	Trypsin/EDTA (10X)	Includes 5 g/L trypsin 1:250 and 2 g/L Versene® (EDTA), manufactured with irradiated porcine trypsin, tested for porcine parvovirus and mycoplasma	-10°C to -20°C	100 mL
17-161E	BE17-161E	Trypsin/EDTA (1X)	Contains 0.5 g/L trypsin 1:250 and 0.2 g/L Versene® (EDTA), manufactured with irradiated porcine trypsin, tested for porcine parvovirus and mycoplasma	-10°C to -20°C	100 mL
17-161F	BE17-161F	Trypsin/EDTA (1X)	Contains 0.5 g/L trypsin 1:250 and 0.2 g/L Versene® (EDTA), manufactured with irradiated porcine trypsin, tested for porcine parvovirus and mycoplasma	-10°C to -20°C	500 mL

Ordering Information – BioWhittaker™ Cell Culture Reagents

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
BE02-034E	BE02-034E	Trypzean™ EDTA (1X)	Recombinant bovine trypsin (NA0)	-10°C to -20°C	100 mL
BE17-605E/U1	**BE17-605E/U1**	UltraGlutamine™ I Supplement, 200 mM (100X)	200 mM Alanyl-L-Glutamine in normal saline. This very stable form of L-Glutamine is used at equimolar concentrations to L-Glutamine and requires little to no adaptive period.	15°C to 30°C	100 mL
	BE17-605E/U1/12	UltraGlutamine™ I Supplement, 200 mM (100X)	200 mM Alanyl-L-Glutamine in normal saline. This very stable form of L-Glutamine is used at equimolar concentrations to L-Glutamine and requires little to no adaptive period.	15°C to 30°C	12 × 100 mL
17-724F	**BE17-724F**	Water for Cell Culture	Water for injection (WFI) quality water is prepared by ultrafiltration, reverse osmosis, deionization, distillation, and sterile filtration	15°C to 30°C	500 mL
17-724Q	**BE17-724Q**	Water for Cell Culture	Water for injection (WFI) quality water is prepared by ultrafiltration, reverse osmosis, deionization, distillation, and sterile filtration	15°C to 30°C	1 L

Growth Factors

Ordering Information –

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
CC-4398	CC-4398	Ascorbic Acid	25.5 mg/mL	-10°C to -20°C	0.5 mL
CC-4098	CC-4098	Bovine Brain Extract	9 mg/mL	-10°C to -20°C	5 mL
CC-4092	CC-4092	Bovine Brain Extract	3 mg/mL	-10°C to -20°C	2 mL
CC-4009	CC-4009	Bovine Pituitary Extract	13 mg/mL	-10°C to -20°C	2 mL
CC-4202	CC-4202	Calcium Chloride	300 mM	15°C to 30°C	2 mL
CC-4107	CC-4107	hEGF Human Epidermal Growth Factor	3 µg/mL	-10°C to -20°C	0.5 mL
CC-4068	CC-4068	hFGF – Human Fibroblastic Growth Factor	1 µg/mL	-10°C to -20°C	1 mL
CC-4205	CC-4205	Human Transferrin	10 mg/mL	-10°C to -20°C	0.5 mL
CC-4323	CC-4323	NSF-1 Neural Survival Factor-1	50X Concentration	-10°C to -20°C	4 mL

Additional Cell Culture Reagents can be found on page 146.

Antibiotics and Antimycotics

Ordering Information – Antibiotics and Antimycotics

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
17-836E	17-836E	Amphotericin B	Contains 250 µg/mL amphotericin B	-10°C to -20°C	100 mL
17-836R	17-836R	Amphotericin B	Contains 250 µg/mL amphotericin B	-10°C to -20°C	20 mL
17-518L	17-518L	Gentamicin Sulfate	50 mg/mL	15°C to 30°C	10 × 10 mL (screw cap vial)
17-518Z	17-518Z	Gentamicin Sulfate	50 mg/mL	15°C to 30°C	1 × 10 mL (screw cap vial)
17-519L	17-519L	Gentamicin Sulfate	10 mg/mL	15°C to 30°C	10 × 10 mL (screw cap vial)
17-519Z	17-519Z	Gentamicin Sulfate	10 mg/mL	15°C to 30°C	1 × 10 mL (screw cap vial)
17-528Z	17-528Z	Gentamicin Sulfate	50 mg/mL	15°C to 30°C	1 × 10 mL (crimp top vial)
	BE02-012E	Gentamicin Sulfate	10 mg/mL	15°C to 30°C	100 mL

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Penicillin-Streptomycin Mixtures

Cat. No.	Size	5,000 Units Penicillin – 5,000 µg Streptomycin	10,000 Units Penicillin – 10,000 µg Streptomycin	20,000 Units Penicillin – 20,000 µg Streptomycin	25,000 Units Penicillin – 25,000 µg Streptomycin	25 µg/mL Amphotericin B	With L-glutamine
17-603E	100 mL	■					
17-602E	100 mL		■				
17-602F	500 mL		■				
17-718R	25 × 4.5 mL				■		■
09-757F	500 mL			■			
17-745H	20 mL		■			■	
17-745E	100 mL		■			■	

-10°C to -20°C

Ordering Information – Penicillin-Streptomycin Mixtures

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
09-757F	09-757F	Penicillin-Streptomycin Mixture	Contains 20,000 units potassium penicillin and 20,000 µg streptomycin sulfate per mL in 0.85% saline	-10°C to -20°C	500 mL
17-602E	DE17-602E	Penicillin-Streptomycin Mixture	Contains 10,000 units potassium penicillin and 10,000 µg streptomycin sulfate per mL in 0.85% saline	-10°C to -20°C	100 mL
17-602F	DE17-602F	Penicillin-Streptomycin Mixture	Contains 10,000 units potassium penicillin and 10,000 µg streptomycin sulfate per mL in 0.85% saline	-10°C to -20°C	500 mL
17-603E	DE17-603E	Penicillin-Streptomycin Mixture	Contains 5,000 units potassium penicillin and 5,000 µg streptomycin sulfate per mL in 0.85% saline	-10°C to -20°C	100 mL
17-745E	17-745E	Penicillin-Streptomycin-Amphotericin B Mixture	Contains 10,000 units potassium penicillin, 10,000 µg streptomycin sulfate and 25 µg Amphotericin B per mL in 0.85% saline	-10°C to -20°C	100 mL
17-745H	17-745H	Penicillin-Streptomycin-Amphotericin B Mixture	Contains 10,000 units potassium penicillin, 10,000 µg streptomycin sulfate and 25 µg Amphotericin B per mL in 0.85% saline	-10°C to -20°C	20 mL
17-718R	17-718R	Penicillin-Streptomycin-L-Glutamine Mixture	Contains 25,000 units potassium penicillin, 25,000 µg streptomycin sulfate and 200mM L-Glutamine	-10°C to -20°C	25 × 4.5 mL

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Buffers and Buffered Salines

Buffers

15°C to 30°C

Ordering Information – Buffers

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
10-548E	10-548E	ACK Lysing Buffer (1X)	Used to Lyse red blood cells in preparations containing white blood cells	15°C to 30°C	100 mL
10-539B	10-539B	Dextrose-Gelatin-Veronal		15°C to 30°C	500 mL

Dulbecco's Phosphate Buffered Saline

15°C to 30°C

Cat. No.	Size	Without Phenol Red	With Calcium and Magnesium	With Glucose	With Sodium Pyruvate
17-513F	500 mL	■	■		
17-513Q	1 L	■	■		
17-512F	500 mL	■			
17-512Q	1 L	■			
04-479Q	1 L	■	■	■	■
17-515F	500 mL (10X)	■			
17-515Q	1 L (10X)	■			

Ordering Information – Dulbecco's Phosphate Buffered Saline

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
17-515F	BE17-515F	Dulbecco's Phosphate Buffered Saline (10X)	95 mM (PO_4) without calcium or magnesium	15°C to 30°C	500 mL
17-515Q	BE17-515Q	Dulbecco's Phosphate Buffered Saline (10X)	95 mM (PO_4) without calcium or magnesium	15°C to 30°C	1 L
04-479Q	04-479Q	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) with calcium, magnesium, 1 g/L glucose and 36 mg/L sodium pyruvate, used in animal embryo transfer procedures	15°C to 30°C	1 L
17-512F	BE17-512F	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) without calcium or magnesium	15°C to 30°C	500 mL
17-512Q	BE17-512Q	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) without calcium or magnesium	15°C to 30°C	1 L
17-513F	BE17-513F	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) with calcium and magnesium	15°C to 30°C	500 mL
17-513Q	BE17-513Q	Dulbecco's Phosphate Buffered Saline (1X)	9.5 mM (PO_4) with calcium and magnesium	15°C to 30°C	1 L

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Buffers and Buffered Salines

Continued

Buffers and Buffered Saline

Cat. No.	Size	Without Phenol Red	Without Calcium or Magnesium	With HEPES
Phosphate Buffered Saline				
17-516F	500 mL	■	■	
17-516Q	1 L	■	■	
17-517Q	1 L	■	■	
BE02-017F	500 mL	■	■	
12-624E	100 mL	■		
17-711E	100 mL	■	■	
UltraSaline A				
12-747F	500 mL	■		■

15°C to 30°C unless noted otherwise in ordering information

Ordering Information – Buffers and Buffered Saline

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
17-737F	17-737F	HEPES Buffer (1 M)	Contains 238.3 g/L HEPES buffer in normal saline	15°C to 30°C	500 mL
17-737E	BE17-737E	HEPES Buffer (1 M)	Contains 238.3 g/L HEPES buffer in normal saline	15°C to 30°C	100 mL
17-517Q	BE17-517Q	Phosphate Buffered Saline (10X)	67 mM (PO_4) without calcium or magnesium	15°C to 30°C	1 L
17-516F	BE17-516F	Phosphate Buffered Saline (1X)	6.7 mM (PO_4) without calcium or magnesium	15°C to 30°C	500 mL
17-516Q	BE17-516Q	Phosphate Buffered Saline (1X)	6.7 mM (PO_4) without calcium or magnesium	15°C to 30°C	1 L
BE02-017F	BE02-017F	Phosphate Buffered Saline EDTA	Used in procedures of generating human dendritic cells from monocytes, pH 7.5 solution	15°C to 30°C	500 mL
12-747F	12-747F	UltraSaline A	HEPES buffered saline without phenol red, enhances trypsin action when used to rinse monolayer before subculture	2°C to 8°C	500 mL
12-624E	12-624E	Veronal Buffer (5X)	Used in serology testing as a diluent or stabilizer	15°C to 30°C	100 mL
17-711E	BE17-711E	Versene® (EDTA), 0.02%	0.2 g/L Ethylenediaminetetraacetic acid (0.53 mM) in DPBS, without calcium or magnesium	15°C to 30°C	100 mL

Viral Serology

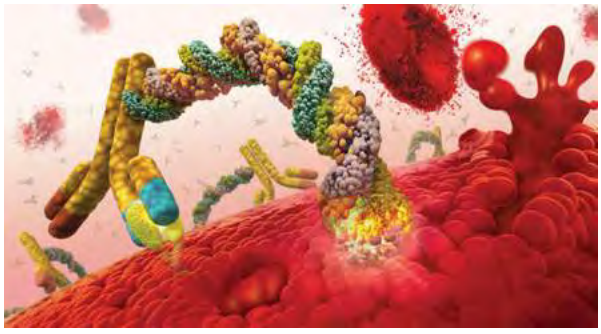
Complement fixation (CF) is an immunological test that can be used to detect the presence of either a specific antibody or a specific antigen. Complement fixation reagents include Guinea Pig Complement, Sheep Erythrocytes, and Hemolysin. The CF test involves two basic principles:

- Complement is irreversibly bound (fixed) to antigen-antibody complexes. The degree of fixation is governed by the relative concentration of either antigen or antibody.
- The lysis of sheep red blood cells in the presence of homologous antibody (hemolysin) is dependent upon the presence of complement.

The complement fixation test is interpreted as follows: antigen + serum + complement + sensitized sheep red blood cells

- antibody present = no hemolysis
- antibody absent = hemolysis

Influenza virus was shown to agglutinate chicken red blood cells (RBC). Subsequently, a variety of viruses have also been shown to agglutinate RBC's from several different species. Viruses have been shown to agglutinate sheep red blood cells, chicken red blood cells, and guinea pig red blood cells in the hemagglutination (HA) assay. It has also been observed that specific antibodies can inhibit hemagglutination which led to the development of the hemagglutination inhibition (HAI) assay. The HA-HAI capability provides a fast and easy method of quantifying both viral antigen and antibody. The specificity and sensitivity of the HAI assay is dependent upon the characteristics of the HA antigen and its interaction with antibody, which will vary with the particular virus under test.



The complement fixation test uses sheep red blood cells (sRBC), pre-bound by anti-sRBC antibody, hemolysin, and serum (usually from a guinea pig) as a source of complement. Complement is a system of serum proteins which interact with the antigen-antibody complex. This reaction causes pores to form in the membrane of the cell which ultimately results in lysis of the red blood cell.

Ordering Information – Viral Serology

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
55-402J	55-402J	Hemolysin	Rabbit anti-sheep erythrocyte serum	4°C	100 mL
30-956J	30-956J	Complement – Guinea Pig	Supplied lyophilized with restoring solution	Lyophilized 4°C	5 mL
30-904J	30-904J	Chicken Red Blood Cells	1 part whole blood, 5 parts Alsever's solution	2°C to 8°C	5 mL
30-957J	30-957J	Guinea Pig Red Blood Cells	1 part whole blood, 5 parts Alsever's solution	2°C to 8°C	5 mL
55-401A	55-401A	Sheep Erythrocytes	40% whole blood, 60% Alsever's solution	2°C to 8°C	100 mL

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Bioprocessing Media and Buffers – Bulk Media

Bioprocessing Media and Buffers – Bulk Media

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Introduction

In January 2013 Sartorius Stedim Biotech and Lonza joined forces to supply high quality, innovative cell culture media and buffers as well as complementary single use solutions for biopharmaceutical, vaccine and cell therapy development and production.

Lonza-Sartorius offers a wide range of liquid and powder media products labeled for further manufacturing along with their expertise to help scale up your projects for antibody, protein and vaccine production as well as for cell therapy. Our products and services include specially engineered media and buffers for dedicated cell lines and USP, DSP and F&F unit operations.

Benefits

- **Assurance of supply** - dual global manufacturing
- **Batch size scalability** - liquid media 1 L to 10,000 L
- Powder media 7 kg to 7,000 kg
- Wide range of primary and secondary packaging to meet specific needs
- Off-the-shelf to fully customized solutions and formulations
- Media formulation options include animal origin (AO) and non-animal origin (NAO), serum-free, protein-free and chemically defined with full traceability to provide solutions for any type of process
- Media formulation options for a broad range of cells e.g. hybridoma, CHO, insect cells, kidney cells, primary cells, stem cells and microorganisms
- Compliant with GMP:820

Long-term collaboration between Lonza and Sartorius to adress all your bioprocessing needs

Selection of different packaging sizes

Volumes as large as 10,000 L

Order online: **www.sartorius.com/media-and-buffers**
North America – Scientific Support: **+1 800 521 0390** (toll free) scientific.support@lonza.com

Sartorius Stedim Biotech Contact Information

For more information on the Sartorius Lonza partnership and products please visit:
www.sartorius.com/media-and-buffers

Sartorius Stedim Biotech Facilities

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Toll-Free +1 800 368 7178 | Fax +1 631 254 4253

Notes

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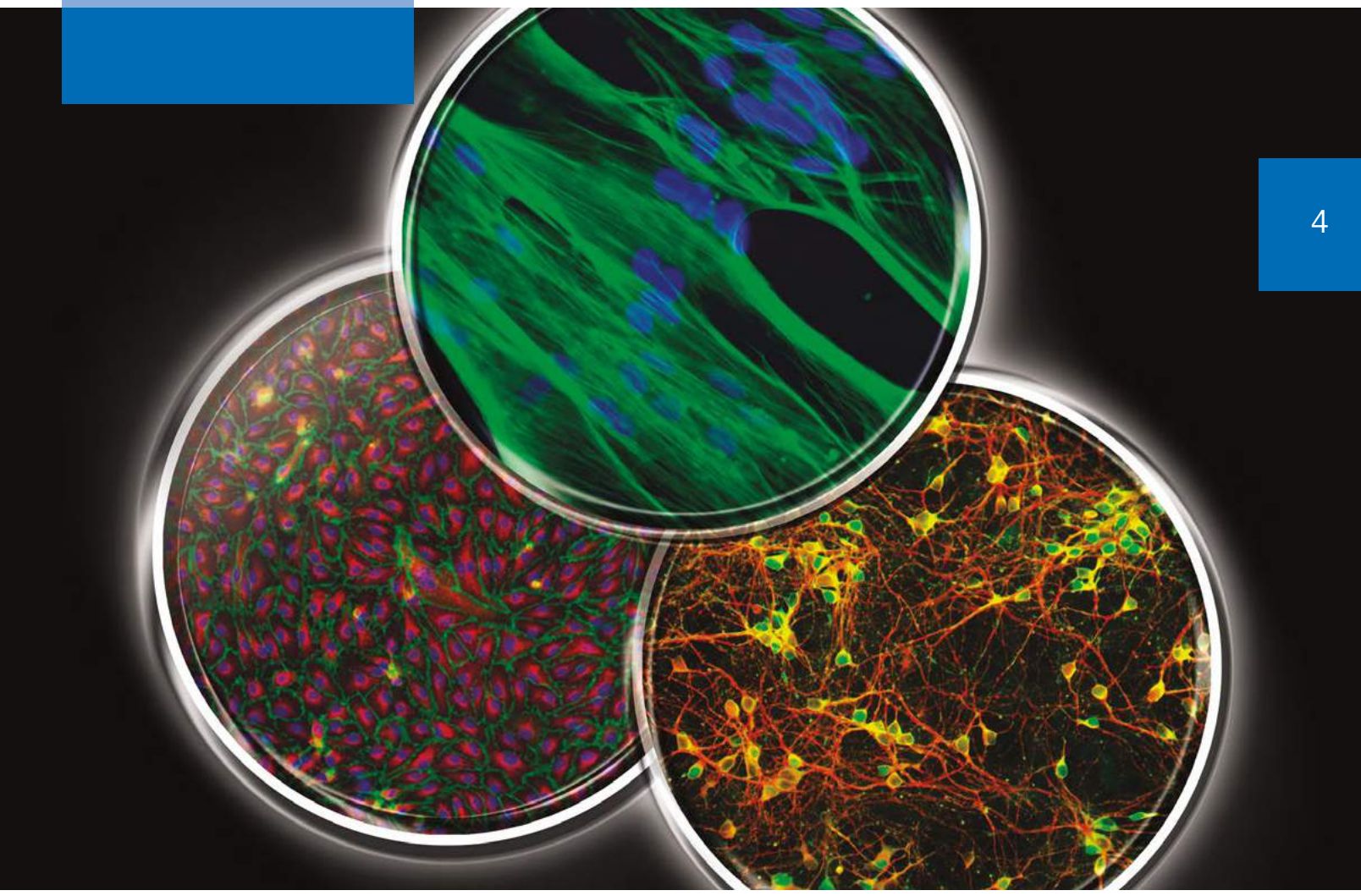
4 Mycoplasma Detection and Prevention



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Mycoplasma Detection and Prevention

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Detection

MycoAlert™ PLUS Mycoplasma Detection Kit 161

Elimination and Prevention

MycoZap™ Mycoplasma Elimination Reagent 163

MycoZap™ Antibiotics 164

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Introduction

One of the most common contaminants present in cell culture laboratories are mycoplasma. A conservative estimate states that between 15–35% of all continuous cell cultures are contaminated with mycoplasma[1], some estimates are even higher (up to 80 % in some countries)[2].

What are Mycoplasma?

- Belong to the family Mollicutes including Mycoplasma, Acholeplasma, Ureaplasma, Entomoplasma, Spiroplasma and other species
- Smallest free-living, self-replicating organisms (size: 0.2 µm–0.8 µm)
- Simple prokaryocytes, lacking a rigid cell wall (surrounded by a single plasma membrane)
- Usually attached to the external surface of the cell membrane
- Relying on their hosts for many nutrients as their biosynthetic capabilities are limited
- Over 180 recognized species
- Six species account for 95% of all mycoplasma infections in cell cultures (*M.orale, M.arginini, M. fermentans, M.salivarum, M.hyorhinis and A.laidlawii*)
- Widespread in nature as parasites of humans, mammals, reptiles, insects and plants

Typical Routes of Mycoplasma Infection in Cell Cultures

- Cross contamination from untested infected cells
- Aerosols created during pipetting
- Using the same bottle of medium for different cell types
- Handling more than 1 cell line in the hood at a time
- Contaminated materials
- Contaminated donor tissue (<1%)
- Direct infection from the researcher

How Insidious Are Mycoplasma?

Contaminations are very difficult to detect or prevent and the presence of mycoplasma can remain undiscovered for months:

- In contrast to bacteria they do not cause visible changes in turbidity or pH
- Not visible under microscopy, even at very high concentrations >10^7 cfu/ml
- Most routine antibiotics used in cell culture are ineffective against mycoplasma
- They are not routinely removed by filtration

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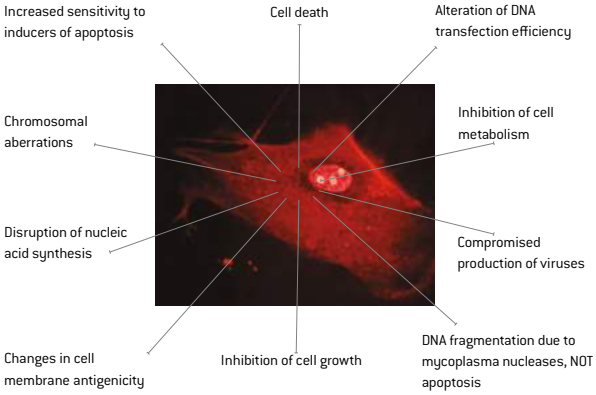
How Do Mycoplasma Affect Your Cells?



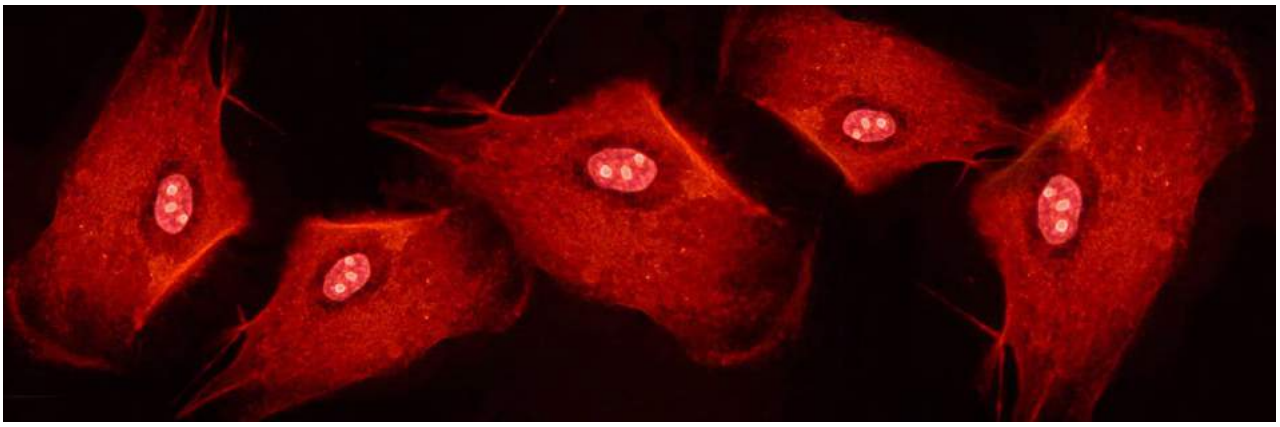
Mycoplasma contamination can seriously impact the reliability, reproducibility, and consistency of experimental results, representing a major problem for basic research as well as for the manufacturing of bioproducts. Standard testing for mycoplasma is an important quality control that should be included in cell culture protocols. We provide a powerful product offering for reliable detection and successful elimination and prevention of mycoplasma contamination:

- **MycoAlert™ Plus Mycoplasma Detection Kit** – Accurate, reliable and universal mycoplasma detection
- **MycoZap™ Mycoplasma Elimination Reagent** – Successful elimination of mycoplasma with low cell toxicity
- **MycoZap™ Prophylactic** – Prevention of mycoplasma contamination in combination with your antibiotic formula of choice
- **MycoZap™ Plus-CL and Plus-PR** – Protection against a broad range of microbial contaminants, such as Gram(+) and Gram(-) bacteria, fungi and mycoplasma

References

1. **Drexler H.G.,** Uphof C.C. (2002): Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology 39: 75–90.
2. **Koshimizu K.,** Kotani H. (1981) in: Procedures for the Isolation and Identification of Human, Animal and Plant Mycoplasmas (Nakamura M., ed.), Saikon, Tokyo, 87-102.

Detection



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Detection

MycoAlert™ PLUS Mycoplasma Detection Kit 161

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MycoAlert™ PLUS Mycoplasma Detection Kit

The MycoAlert™ Plus Assay is a selective biochemical test that exploits the activity of mycoplasmal enzymes which are found in all six of the main mycoplasma cell culture contaminants and the vast majority of 180 mycoplasma species, but are not present in eukaryotic cells. Viable mycoplasma in a test sample are lysed and the enzymes react with the MycoAlert™ PLUS Substrate, catalyzing the conversion of ADP to ATP. By measuring the level of ATP in a sample via a luciferase reaction, both before (read A) and after the addition of the MycoAlert™ PLUS Substrate (read B), a ratio can be obtained which is indicative of the presence or absence of mycoplasma. The MycoAlert™ PLUS Assay generates a strong light signal providing broad compatibility with plate luminometers and multifunctional readers.

Benefits

- Results in <20 minutes by a simple 2-step assay
- Bioluminescence-based technology – no DNA extraction necessary
- Convenient enzymatic assay control available for monitoring system performance

Applications

- Detects all common mycoplasma and acholeplasma contaminations
- Suited for cell culture screening in research environment
- Suited for testing of fresh media, supplements or water

2°C – 8°C, do not freeze prior to reconstruction

www.lonza.com/mycoalert

Simple Protocol for Single Tube or 96-well Format

Cell culture supernatant (100 µl)

▼

Add MycoAlert™ PLUS Reagent (Wait 5 min)

▼

Measure luminescence (Reading A)

▼

Add MycoAlert™ PLUS Substrate (Incubate 10 min)

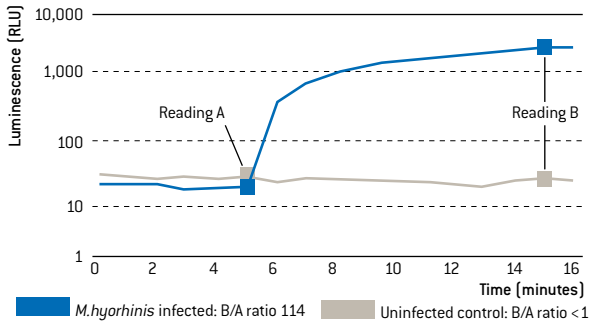
▼

Measure luminescence (Reading B)

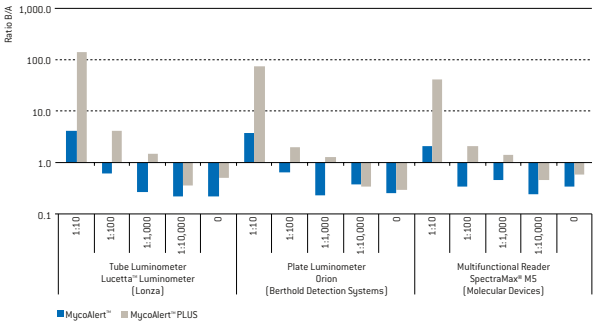
▼

Ratio B/A >1 equals mycoplasma contamination

Assay Principle



Kinetics of light emission for uninfected and infected cells. The B/A ratio indicates the presence or absence of mycoplasma.



Comparison of MycoAlert™ and MycoAlert™ PLUS Assay. Dilution series of MycoAlert™ Assay Control demonstrates the increased sensitivity of various luminometers when using MycoAlert™ PLUS Assay.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Size
LT07-701	LT07-701	MycoAlert™ PLUS Mycoplasma Detection Kit	10 reactions
LT07-703	LT07-703	MycoAlert™ PLUS Mycoplasma Detection Kit	30 reactions
LT07-705	LT07-705	MycoAlert™ PLUS Mycoplasma Detection Kit	50 reactions
LT07-710	LT07-710	MycoAlert™ PLUS Mycoplasma Detection Kit	100 reactions
LT27-292	LT27-292	MycoAlert™ PLUS Assay Buffer	20 mL
LT07-518	LT07-518	MycoAlert™ Assay Control Set	10 tests

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Elimination and Prevention

Elimination and Prevention

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MycoZap™ Mycoplasma Elimination Reagent

The MycoZap™ Reagent can eliminate detectable mycoplasma contamination in as few as 4 days and has been optimized to clear mycoplasma with minimal toxic effects on the infected cells. It eliminates mycoplasma by using a combination of antibiotic and antimetabolic agents. This approach allows for a highly reliable elimination of mycoplasma that cannot be achieved by the use of antibiotics alone. The MycoZap™ Reagent can be used to eradicate mollicutes, including Mycoplasma, Acholeplasma, Spiroplasma and Entomoplasma species in cell cultures.

Benefits

- Efficient mollicute elimination by a combination of antibiotic and antimetabolic agents
- Minimal toxic effects on cells

Applications

- Eradicates Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma
- Suited for a broad range of cell cultures

2°C to 8°C

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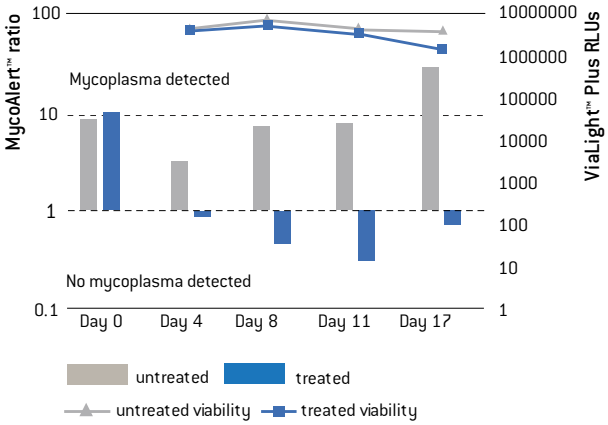
Efficient Mycoplasma Removal with Minimal Effect on Cell Viability



The MycoZap™ Reagent treatment eliminates mycoplasma in as few as 4 days (detected by MycoAlert™ Assay) with minimal impact on cell viability (determined by ViaLight™ Assay).

Ordering Information – Reagent

Cat. No. NA	Cat. No. EU	Product Name	Size
LT07-818	LT07-818	MycoZap™ Mycoplasma Elimination Reagent	1 treatment, for T-25 flask
LT07-918	LT07-918	MycoZap™ Mycoplasma Elimination Reagent	5 treatments, for T-25 flask

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MycoZap™ Antibiotics

MycoZap™ Antibiotics are extremely powerful combinations of innovative antibiotics for the protection of cell cultures from mycoplasma contamination. While MycoZap™ Prophylactic prevents mycoplasma contamination, MycoZap™ Plus offers complete protection against a broad range of common contaminants including mycoplasma.

MycoZap™ Prophylactic

Benefits

- Specifically prevents mycoplasma contamination
- Also active against other species of the mycoplasma group like Acholeplasma and Spiroplasma

Applications

- Can be used in combination with other antibiotics (e.g., Pen/Strep) to prevent other microbial contaminants

MycoZap™ Plus-CL and MycoZap™ Plus-PR

Benefits

- Active against mycoplasma, Gram(-) and Gram(+) bacteria as well as yeast and fungi
- Complete solution replacing Pen/Strep formulation

Applications

- MycoZap™ Plus-CL for protection of cell lines
- MycoZap™ Plus-PR optimized for gentle protection of primary cells

Immediate use: 2°C to 8°C
Long-term storage: below -18°C

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	Mycoplasma Only Solution	Complete Solutions	
	MycoZap™ Prophylactic	MycoZap™ Plus-CL	MycoZap™ Plus-PR
Prevention against mycoplasma	■	■	■
Prevention against	No; but can be used in combination with other antibiotic formula of choice		
– Gram(+) bacteria		■	■
– Gram(-) bacteria		■	■
– Fungi		■	■
– Yeast		■	■
Suited for primary cells	■		■
Suited for cell lines	■	■	

Ordering Information – Reagent

Cat. No. NA	Cat. No. EU	Product Name	Size
VZA-2011	VZA-2011	MycoZap™ Plus-CL Antibiotic	10 × 1 mL
VZA-2012	VZA-2012	MycoZap™ Plus-CL Antibiotic	1 × 20 mL
VZA-2021	VZA-2021	MycoZap™ Plus-PR Antibiotic	10 × 1 mL
VZA-2022	VZA-2022	MycoZap™ Plus-PR Antibiotic	1 × 20 mL
VZA-2031	VZA-2031	MycoZap™ Prophylactic	10 × 1 mL
VZA-2032	VZA-2032	MycoZap™ Prophylactic	1 × 20 mL

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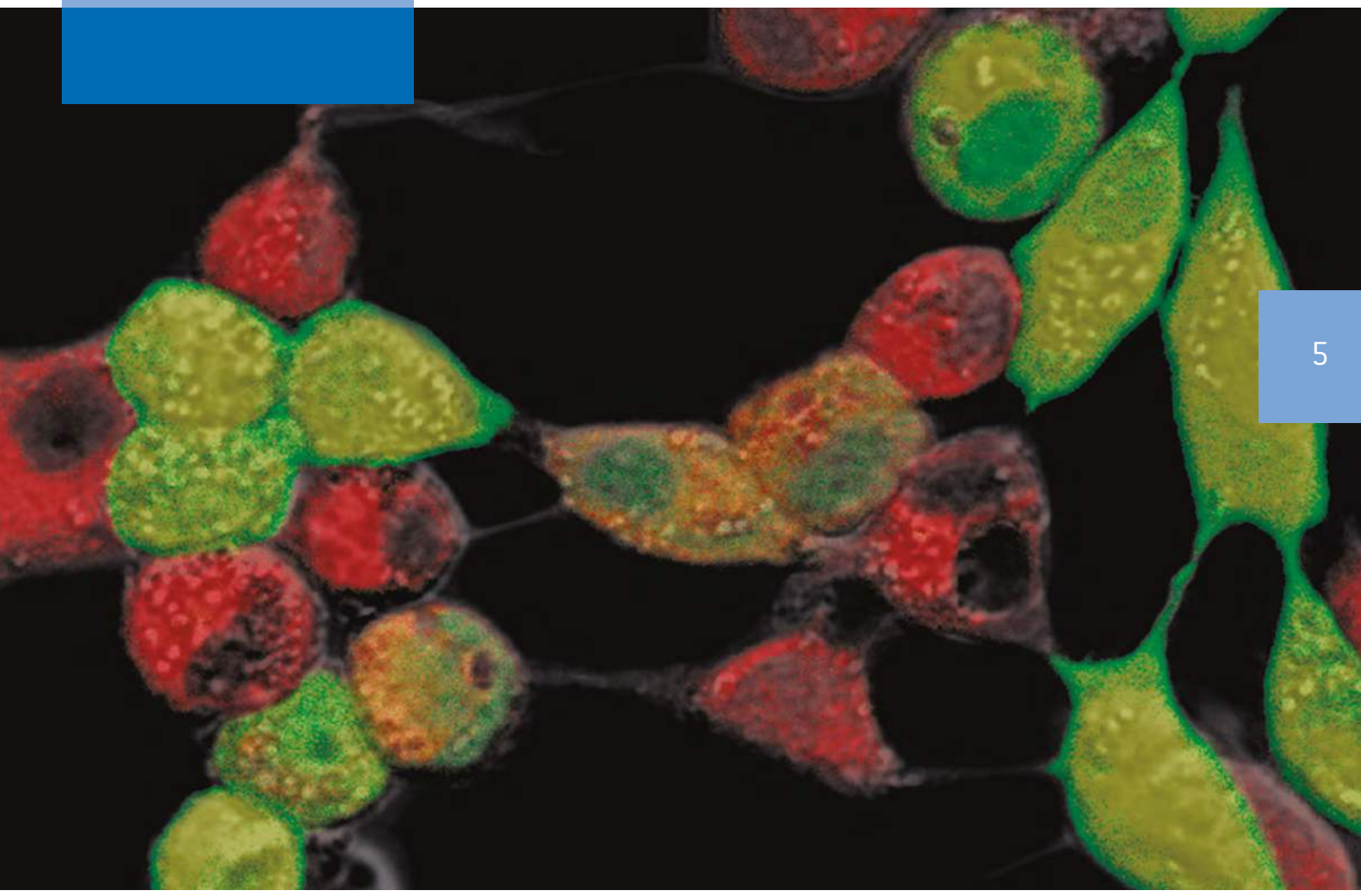
5 Transfection



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Transfection

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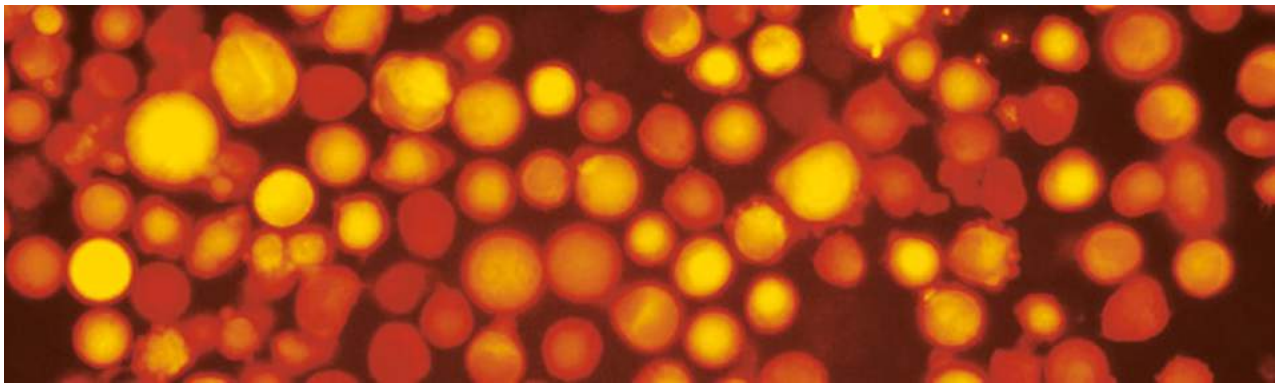
5

Nucleofector™ Technology



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Introduction

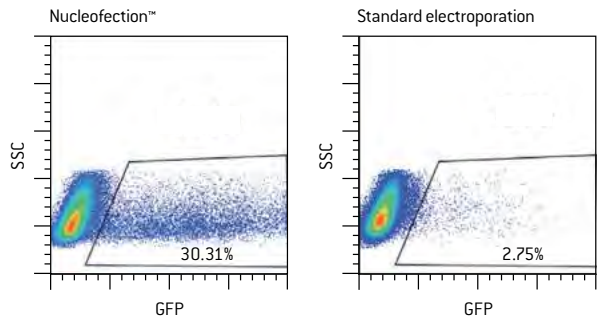
The application of systems biology and multidisciplinary approaches require that cells and model systems display *in vivo* like cellular functionality. This means that the future of cell transfection is in using primary cell types, and that transfecting these physiologically relevant cell types is typically a very difficult task using traditional methods. Additionally, when using relevant cell lines as model systems, the critical issues are to achieve reproducibly efficient transfection with high levels of viability while matching throughput capability with the number of transfections required at each project phase – from proof of concept, through to scale-up and screening-like approaches. With the **Nucleofector™ Technology** primary cells and stem cells, as well as cell lines, can be consistently transfected at high efficiency.

Developed in 1998, the Nucleofector™ Technology was introduced to the research market in 2001 as the first efficient non-viral transfection method for primary cells and hard-to-transfect cell lines. Since then the technology has evolved through constant innovation.

The Principle

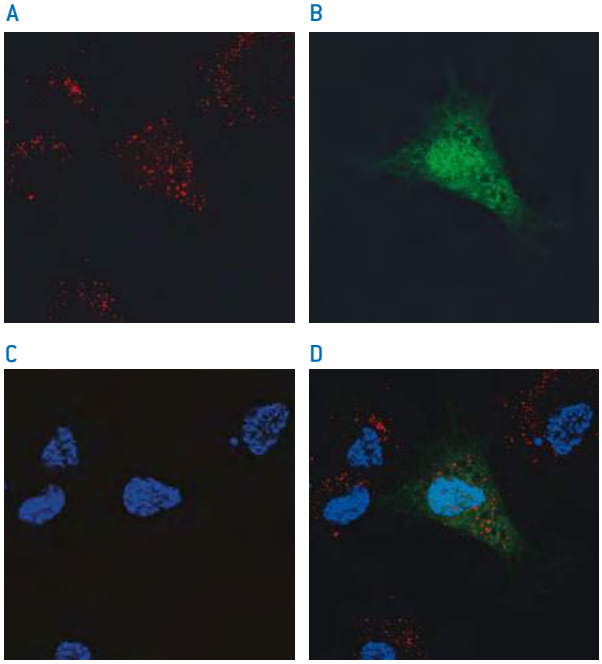
Nucleofection is a technology based on the momentary creation of small pores in cell membranes by applying an electrical pulse. The comprehensive way in which Nucleofector™ Programs and cell-type specific solutions are developed enables nucleic acid substrate delivery not only to the cytoplasm, but also through the nuclear membrane and into the nucleus. This allows for high transfection efficiencies up to 99% and makes the transfection success independent from cell proliferation.

Nucleofector™ Technology – the Superior Non-viral Method



Nucleofector™ Technology – the superior non-viral method. Transfection of the human natural killer cell line NKL using traditional electroporation and Nucleofection. 5 × 10^6 NKL cells were transfected with 2.5 µg of pmaxGFP™ Vector. Nucleofection: Nucleofector™ Solution V; Program O-017. Standard electroporation: 25 mV, 96 µF. Transfection efficiency was monitored by flow cytometry after 24 hours. Cells transfected by Nucleofection show a significantly better transfection efficiency compared to cells transfected by traditional electroporation. Cell viability, as measured 18 hours after transfection was also superior using Nucleofection. (Data courtesy of Dr. John Coligan, Laboratory of Immunogenetics, NIH/NIAID, Rockville, MD, USA. *J Immunol Methods* (2004) **284**: 133-140.)

DNA Delivery Straight Into the Nucleus



DNA delivery straight into the nucleus. Normal human dermal fibroblasts (neonatal) were transfected with 2.5 µg ™R-labeled plasmid DNA encoding eGFP. After 2 hours, cells were fixed with 3.5% PFA and analyzed by confocal microscopy. ™R label is shown in (A), GFP fluorescence in (B), DAPI nuclear staining in (C) and a merge of all 3 fluorescent labels in (D).

Introduction

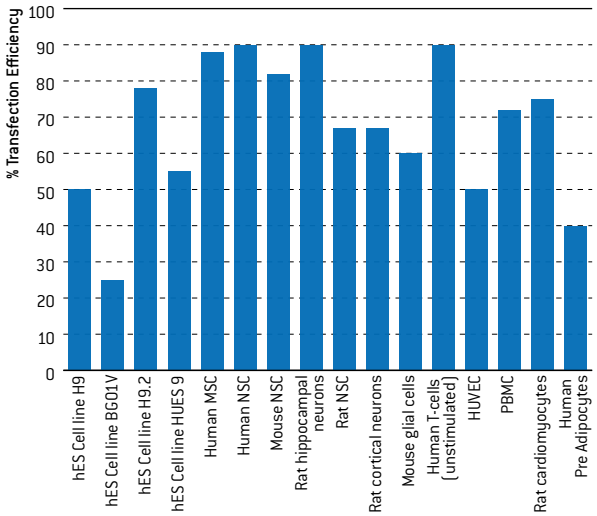
Continued

What Benefits are Important for Your Work?

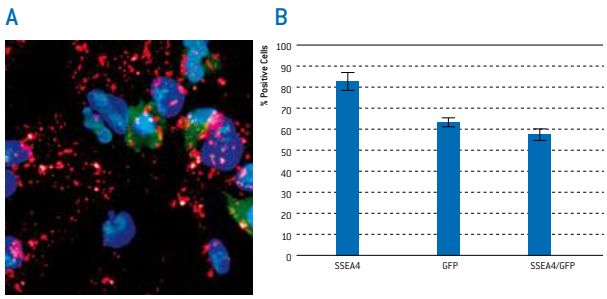
- **Superior transfection performance?**
  - Electrical parameters are optimized to gain high transfection efficiency and retain highest viability
  - Excellent preservation of the physiological status of transfected cells
- **Easy-to-use technology?**
  - More than 650 cell-type specific protocols lead to direct transfection success with a multitude of different cell types
  - Easy optimization protocols for cell lines and primary cells allow for quick and streamlined optimization of virtually any cell type
  - Dedicated White Papers support numerous applications, such as siRNA transfection and transfection of neurons
  - Excellent technical and applicative support?
- **Highly-skilled Scientific Support Team to assist you in your research**
  - Scientific Support Team members have a masters or PhD level education in biology, biochemistry or biotechnology
  - Many of them with over 10 years experience in transfection support
- **Proven and innovative technology?**
  - More than 4000 peer-reviewed publications and thousands of systems placed worldwide
- **Modularity of the 4D-Nucleofector™ System allows easy adaptation to new applications**
  - Invention of Nucleofection of cells in adherence
- **Using various cell numbers for different applications?**
  - Nucleofection of 2×10^4 to 2×10^7 cells are feasible within one single device
  - Transferability of protocol conditions from small to larger cell numbers with the new 4D-Nucleofector™ System
- **Easy expansion of your research?**
  - Explore complex systems by using the same conditions to deliver DNA, RNA, oligonucleotides, PNA, peptides, or proteins
  - Different device platforms fulfill your choice of sample throughput from 1 through 384 transfections per run including automated high-throughput
- **Avoiding cross-contamination?**
  - Disposable, sterile Nucleofection Vessels minimize the risk of cross-contamination with cell or substrate leftovers

www.lonza.com/celldatabase

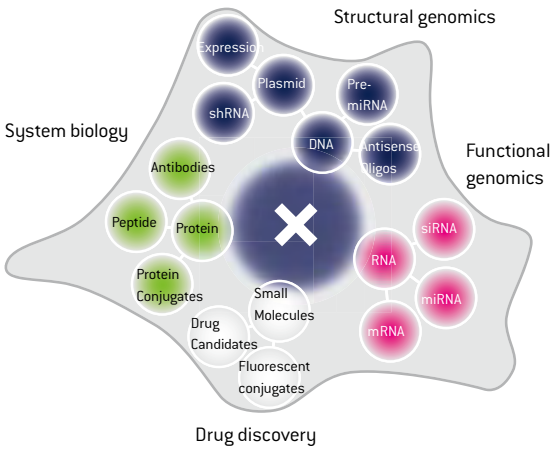
www.lonza.com/citations



Exemplary transfection efficiency data for primary cells and human stem cells.



Conserving functionality – the first step to meaningful results. Human H9 ES cells preserve pluripotency post Nucleofection. H9 cells were transfected by Nucleofection with the pmaxGFP™ Vector. (A) Cells analyzed after 24 hours show expression of GFP (green) as well as of the pluripotency markers SSEA4 (red) and Oct4 (purple). The blue signals refer to nuclear staining by DAPI. (B) The percentage of double-positive cells (GFP/SSEA) was analyzed by flow cytometry. (Data kindly provided by Jennifer Moore, Rutgers University, Piscataway, USA.)



Nucleofector™ Technology – delivers the widest range of substrates. Overview of substrates that can be transfected into primary cells and cell lines using Nucleofection.

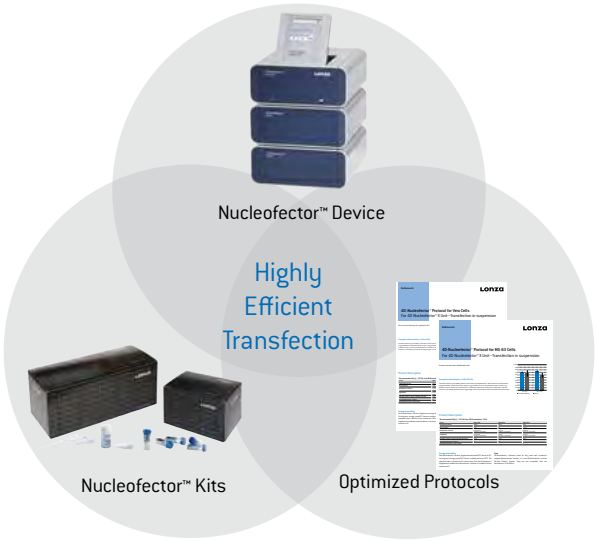
Components of the Nucleofector™ Technology

The Nucleofector™ Technology relies on the combination of a Nucleofector™ Device and cell specific Nucleofector™ Kits:

– **The Nucleofector™ Device** delivers unique electrical parameters. The electrical settings are pre-programmed for each optimized cell type and can be selected via the device or PC software. We offer three different device platforms plus an add-on device (see table below)

– **The Nucleofector™ Kits** contain a specific Nucleofector™ Solution and Supplement, specified cuvettes, pipettes, and the pmaxGFP™ Control Vector. All Nucleofector™ Solutions provide a protective environment that allows for high transfection efficiency and cell viability, while helping to maintain physiologically relevant cellular functions. A collection of Nucleofector™ Kits with optimized protocols for primary cells and cell lines is available

– Besides providing optimal Nucleofection Conditions, **Optimized Protocols** offer comprehensive guidance, including tips for cell sourcing, passage, growth conditions and media, and post transfection culture



Overview of Nucleofection Platforms

	Advanced Platform	96-well Add-on	High-throughput Platform	Basic Device
Device	**4D-Nucleofector™ System**	**96-well Shuttle™ Device**	**384-well Nucleofector™ System**	**Nucleofector™ 2b Device**
Throughput (samples per run)	Low to medium (1-16)	Low to high (1-96)	High (384)	Low (1)
Reaction volume	20 µL + 100 µL	20 µL	20 µL	100 µL
Electrode material	Conductive polymer	Conductive polymer	Conductive polymer	Aluminum
Low cell numbers (20 µL)	2×10^4 to 1×10^6	2×10^4 to 1×10^6	2×10^4 to 1×10^6	–
High cell numbers (100 µL)	2×10^5 to 2×10^7	–	–	2×10^5 to 2×10^7
DNA Vector amount/sample	0.2–1 µg (20 µL) 1–5 µg (100 µL)	0.2–1 µg	0.2–1 µg	1–5 µg
siRNA amount/sample (concentration 2 nM – 2 µM)	0.04–40 pmol (20 µL) 0.2–200 pmol (100 µL)	0.04–40 pmol	0.04–40 pmol	0.2–200 pmol
Adherent Nucleofection	■	–	–	–
Compatibility with 96-well Shuttle™ Device	■	–	–	–

Advanced Platform: 4D-Nucleofector™ System

Based on user feedback, our engineers and scientists have developed the new innovative 4D-Nucleofector™ System. This system is designed for maximum flexibility and enables Nucleofection of cells in several formats combined with advanced performance and convenience. Due to its modular design the 4D-Nucleofector™ System is extremely flexible in regard to the supported applications.
The operation software allows you to design and save individual experimental setups. Additionally, a PC editor is available that enables predefinition of experiments on a PC which can then be uploaded to the 4D-Nucleofector™ Core Unit via the integrated USB port.

Hardware and Software Components

The 4D-Nucleofector™ System is a modular system comprising of one **Core Unit** and the **X Unit** and **Y Unit** as the first available functional units:

- **Core Unit** – Controlling the 4D-Nucleofector™ System
- **X Unit** – Supporting Nucleofection of various cell numbers in different formats
- **Y Unit** – Enabling adherent Nucleofection in 24-well culture plates

For ordering information and further details, please refer to page 175.

4D-Nucleofector™ System

What Benefits are Important for Your Work?

Using different cell numbers for different applications?

- Same protocol for 100 µL and 20 µL transfection volume
- 100 µL Nucleocuvette™ for high cell numbers up to 2×10^7
- 20 µL Nucleocuvette™ Strip for low cell numbers down to 2×10^4

Working with various throughputs?

- Flexible throughput from 1 to 16 samples
- Parallel processing of one or two 100 µL Nucleocuvettes™
- Pre-programming of settings for up to 50 single 100 µL Nucleocuvettes™ or one 20 µL Nucleocuvette™ Strip
- Kit costs tailored to your throughput

Transfecting different primary cell types?

- Five primary cell kits covering a broad range of primary cells
- New Primary Cell Optimization Kit for cells lacking an optimized protocol
- Easy optimization of a variety of cell types using the 96-well Shuttle™ Add-on System

Preserving cell functionality?

- Adherent Nucleofection of neurons at later developmental stages
- No release of metal ions due to conductive polymer electrodes

Adherent Nucleofection

Electroporation-based methods have so far required cells to be in suspension for transfection. The Nucleofector™ Technology entered a new era and allows direct Nucleofection of cells in adherence. Cells which typically grow in adherence in cell culture, can be kept and transfected by Nucleofection in their physiological state.

The Y Unit of the 4D-Nucleofector™ System works with disposable conductive polymer Dipping Electrode Arrays that can be inserted into standard 24-well culture plates for Nucleofection.

Benefits

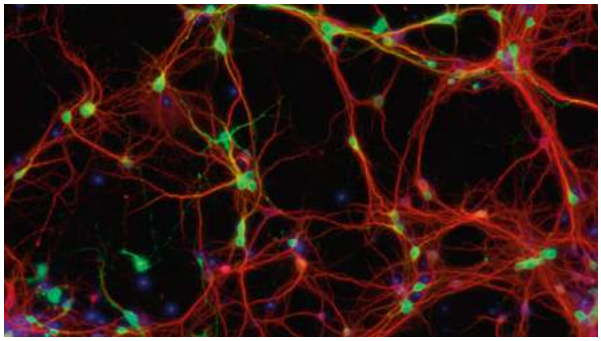
- Pre- and post Nucleofection culture in 24-well culture plates
- Nucleofection of cells at any time point during this culture period, i.e. at a later developmental stage
- Transfection efficiencies up to 70% combined with high viabilities
- Compatible with Clonetics™ Primary Animal Neurons

Applications

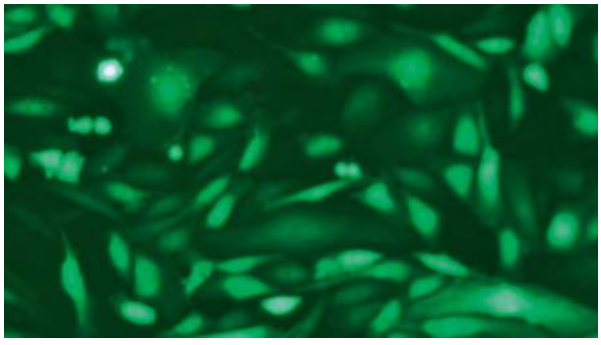
- Enables Nucleofection of cells in adherence in 24-well culture plates

For ordering information and further details, please refer to pages 175 and 184.

www.lonza.com/adherent-nucleofection



Efficient adherent Nucleofection of neurons in 24-well culture plates. Mouse cortical neurons were seeded into poly-D-lysine coated 24-well plates (1 × 10^5 cells/well). After 6 DIV, cells were transfected with pmaxGFP™ Vector using the AD1 4D-Nucleofector™ Y Kit. One day post Nucleofection, cells were stained by MAP2 antibody (red) and analyzed by fluorescence microscopy for maxGFP™ protein expression.



Efficient adherent Nucleofection of endothelial cells in 24-well culture plates. Human umbilical vein endothelial cells (HUVEC) were isolated and plated in passage 1 into collagen-coated 24-well plates at a density of 50,000 cells/well. After 1DIV cells were transfected with 16 µg pmaxGFP™ Vector using AD1 4D-Nucleofector™ Y Solution and program CA-215. Cells were analyzed for maxGFP™ Protein expression after 24h. (Data kindly provided by M. Sauvage, Pharmaceutical Industry, FR)

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4D-Nucleofector™ System – Higher Quality Standards

For the 4D-Nucleofector™ System, Lonza offers accessory products which provide higher quality standards for transfection applications in upstream GMP manufacturing environments.

4D-Nucleofector™ LogWare

Benefits

- Compliance with Title 21 CFR part 11/annex 11
- User administration
- Electronic signatures with user name and password
- Logging of any modification, creation of data or user interaction with time stamp
- Reporting of result failures with failure description
- Data export according to Title 21 CFR part 11
- Generation of audit trails
- No data deletion possible

GMP Solution 4D-Nucleofector™ Kits

Benefits

- Animal origin-free formulation
- Solutions produced under GMP conditions
- Certified for absence of DNA/RNA, DNase/RNase, particles and endotoxins
- CoA for Nucleofector™ Solutions, Supplements and Nucleocuvette™ Vessels
- No metal ion release during Nucleofection
- Validation of ethylene oxid sterilization
- Tested for biocompatibility

Applications

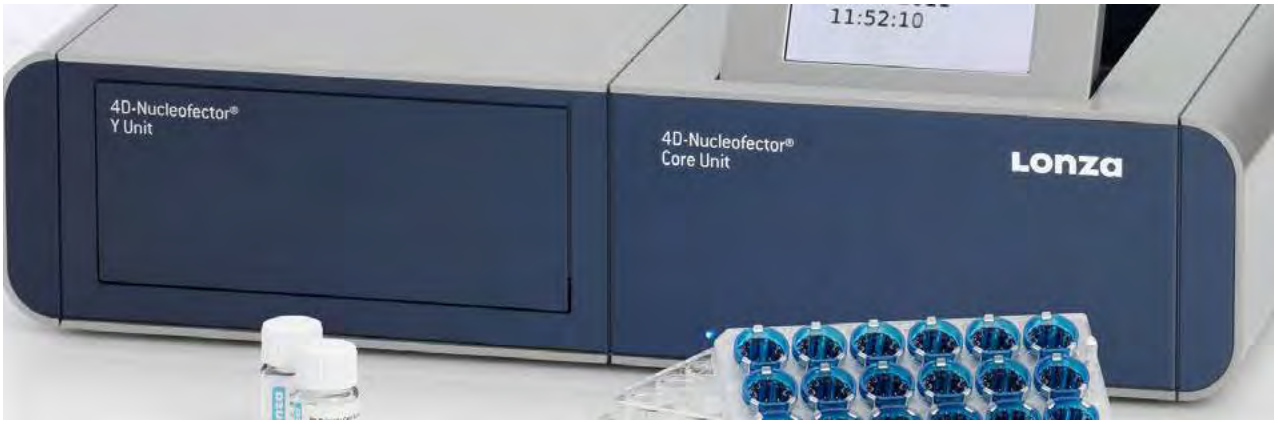
- Transfection experiments under certified animal component-free conditions
- Applications requiring higher quality standards
- Generation of induced pluripotent stem cells (iPSCs)
- Efficient transfection of cell lines relevant to biopharmaceutical protein production, e.g., CHO, suspension CHO, suspension HEK293

Ordering Information

Cat. No. NA	Cat. No. EU	Product Name	Size
SAAF-1001	SAAF-1001	4D-Nucleofector™ LogWare	
V4XPG-3024	V4XPG-3024	cGMP Solution P3 Primary Cell 4D-Nucleofector™ Kit	24 reactions (100 µL Nucleocuvette™ Vessel)
V4XCG-2024	V4XCG-2024	cGMP Solution SF Primary Cell 4D-Nucleofector™ Kit	24 reactions (100 µL Nucleocuvette™ Vessel)

NOTE: Nucleofector™ Kits and Devices are for research use only and are not intended for use in humans.

Nucleofector™ Devices and Systems



Nucleofector™ Devices and Systems

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Nucleofector™ 2b Device	177
384-well Nucleofector™ System	178

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4D-Nucleofector™ System

The 4D-Nucleofector™ System is a modular system comprising one **Core Unit** and the **X Unit** and/or **Y Unit** as the first available functional units, each suited for different applications.

The Core Unit

Benefits

- Controls up to five functional units
- 5.7" foldable touch screen to operate the system
- Intuitive operation software for designing and saving individual experimental setups
- USB port for software update and data transfer
- Comprises USB and serial connectivity for the 96-well Shuttle™ Add-on

Applications

- Controlling the 4D-Nucleofector™ System

The X Unit

Benefits

- Features positions for 20 µL Nucleocuvette™ Strips and 100 µL single Nucleocuvette™
- Seamless transfer of conditions between different Nucleofection Vessels
- Comprises HV connectivity for the 96-well Shuttle™ System
- Electrically driven drawer for cuvette retainer

Applications

- Supporting Nucleofection of various cell numbers in different formats

Technical Specifications	
Dimensions (w × d × h)	24.5 × 10.5 × 28 cm (9.7 × 4.1 × 11.0 in) System comprising Core Unit and one functional Unit assembled side-by-side
Weight	8.0 kg (17.8 lb) System comprising Core Unit and one functional Unit
Power supply	100–110 VAC or 230 VAC, 50–60 Hz, self-regulating
Power consumption	140 VA
Protection	IP 20

The Y Unit

Benefits

- Features positions for one 24-well culture plate with inserted Dipping Electrode Array
- Electrically driven drawer for plate retainer

Applications

- Enables Nucleofection of pre-plated cells in adherence in 24-well culture plates

www.lonza.com/4d-nucleofector

Ordering Information – Devices

Cat. No. NA	Cat. No. EU	Product Name	Product Description
AAF-1002B	AAF-1002B	4D-Nucleofector™ Core Unit	
AAF-1002X	AAF-1002X	4D-Nucleofector™ X Unit	Requires the Core Unit to build complete system
AAF-1002Y	AAF-1002Y	4D-Nucleofector™ Y Unit	Requires the Core Unit to build complete system
AWC-1001	AWC-1001	4D-Nucleofector™ Service Contract	Valid for 1 year, can be purchased at any time during the guarantee period. Comprises diagnosis and repair of the device including all replacement parts and costs for return shipments of repaired device.
AWE-1002	AWE-1002	4D-Nucleofector™ Guarantee Extension	Valid for 2 years, must be purchased together with the system.

96-well Shuttle™ System

The 96-well Shuttle™ System is a medium-throughput add on for the 4D-Nucleofector™ System suited for convenient optimization of Nucleofection Conditions or as an assay establishment tool. The complete system consists of three components:

- The **4D-Nucleofector™ System** (Core Unit and X Unit) serving as the program delivery unit
- The **96-well Shuttle™ System** which mediates the transfer of the respective 96-well program to a specific well of the 96-well Nucleocuvette™ Plate
- A **laptop computer** with the 96-well Shuttle™ Software controlling the interaction between the devices

Benefits

- Up to 96 independent programs can be run per plate, processed automatically in <5 minutes
- Modular 6 × 16 Nucleocuvette™ Plate for scalable throughput
- Fulfills pre-requisites for liquid handling integration

Applications

- Optimization of any difficult-to-transfect cell type in just 1 plate
- Variable cell numbers from 10^4–10^6 cells per reaction

Technical Specifications	
Dimensions (w × d × h)	34 × 27 × 10 cm (13.39 × 10.63 × 3.94 in)
Weight	3.0 kg (6.6 lb)
Power supply	110 VAC +10%/-20% or 230 VAC +10%/-20% 50–60 Hz, self–regulating
Power consumption	20 VA
Protection	IP 22

Nucleofector™ 96-well Shuttle™ System

Ordering Information – Devices

Cat. No. NA	Cat. No. EU	Product Name	Product Description
AAM-1001S	AAM-1001S	96-well Shuttle™ Device	Including Laptop and Nucleofector™ 96-well Shuttle™ Software, 4D-Nucleofector™ Core and X Unit must be purchased separately
AWM-1002	AWM-1002	96-well Shuttle™ Guarantee Extension	Valid for 2 years, must be purchased together with the system.
AWB-1001	AWB-1001	96-well Shuttle™ Service Contract	Valid for 1 year, can be purchased at any time during the guarantee period. Comprises diagnosis and repair of the system including all replacement parts and costs for return shipments of repaired system
AAF-1002B	AAF-1002B	4D-Nucleofector™ Core Unit	
AAF-1002X	AAF-1002X	4D-Nucleofector™ X Unit	Requires the Core Unit to build complete system

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Nucleofector™ 2b Device

The Nucleofector™ Device is the single cuvette based system that has been used in research labs since 2001. It allows efficient transfection of hard-to-transfect cell lines and primary cells with different substrates (e.g., DNA vectors or siRNA oligonucleotides) in low-throughput format. The Nucleofector™ II/2b Device can also be used for bacteria transformation by using alternative cuvettes.

Benefits

- Highly efficient transfection of primary cells and cell lines
- Reliable results due to high viability and preservation of cell functionality
- Over 150 ready-to-use Optimized Protocols containing cell-type specific guidance

Applications

- Low-throughput transfection in single cuvette format
- Transfection of plasmid DNA, siRNA, shRNA, miRNA, RNA and more, e.g., Morpholinos
- Transfection of peptides, proteins or small molecules
- Approaching 4,000 peer-reviewed publications
- Suited for bacteria transformation

Technical Specifications	
Dimensions (w × d × h)	30 × 23 × 11 cm (11.81 × 9.06 × 4.33 in)
Weight	2.8 kg (6.2 lb)
Power supply	100–110 VAC or 230 VAC 50–60 Hz, self-regulating
Power consumption	50 VA/fuse T630mA L250V
Protection	IP 20, EN 61010-1, UL 61010A-1

www.lonza.com/protocols

Ordering Information – Devices

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
AAB-1001	AAB-1001	Nucleofector™ 2b Device		
AWD-2002	AWD-2002	Nucleofector™ 2b Guarantee Extension	Valid for 2 years, must be purchased together with the system.	
AWA-2001	AWA-2001	Nucleofector™ 2b Service Contract	Valid for 1 year, can be purchased at any time during the guarantee period. Comprises diagnosis and repair of the device including all replacement parts and costs for return shipments of repaired device.	
VKA-1001	VKA-1001	Electroporation Cuvettes for Bacteria (1 mm gap)		50 cuvettes

384-well Nucleofector™ System

The new 384-well Nucleofector™ System is an independent platform for high-throughput Nucleofection in a 384-well format. With an extremely fast plate processing time of 1 minute per plate, it is perfectly suited for screening applications with maximum reproducibility.
The 384-well Nucleofector™ System consists of a Power Supply Unit generating the high voltage pulses, a Plate Handler Unit and an intuitive PC-based Operation Software. The 384-well Nucleofector™ Kits use existing 96-well Shuttle™ Protocols with newly developed conductive polymer 384-well Nucleocuvette™ Plates. For an automated Nucleofection Process that requires long-term storage of cells in Nucleofector™ Solution, some cells may require specialized Automation Kits.

Benefits

– Processes a 384-well plate in 1 minute
– Uses existing 96-well Shuttle™ Protocols
– Intuitive PC-based Operation Software

Applications

– High-throughput Nucleofection of low cell numbers down to 2 × 10^4 cells
– Seamless integration into automated liquid handling environments

Contact Scientific Support for exact guidance regarding optimal kit use.

Power Supply Unit (left), and Plate Handler Unit (right), with loaded 384-well Nucleocuvette™ Plate

Technical Specifications	
Dimensions (w × d × h)	384-well Nucleofector™ Plate Handler: 40 cm × 42 cm × 15 cm (15.7 × 16.5 × 5.9 in)
	384-well Nucleofector™ Power Supply: 13.5 cm × 50 cm × 45 cm (5.3 × 19.6 × 17.7 in)
Weight	384-well Nucleofector™ Plate Handler: 10 kg (22.04 lb)
	384-well Nucleofector™ Power Supply: 14 kg (30.86 lb)

Ordering Information – Devices

Cat. No. NA	Cat. No. EU	Product Name	Product Description
AAU-1001	AAU-1001	384-well Nucleofector™ System	Includes power supply, plate handler, laptop, and software
AWU-1001	AWU-1001	384-well Nucleofector™ Service Contract	Valid for 1 year, can be purchased at any time during the guarantee period. Comprises diagnosis and repair of the system along with all replacement parts.
AWT-1001	AWT-1001	384-well Nucleofector™ System Installation and Training	

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Nucleofector™ Kits

Nucleofector™ Kits

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Primary Cell Kits for 4D-Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

With our new conductive polymer cuvette concept, which was first established for the 96-well Shuttle™ System and now transferred to the new platforms, we were able to streamline our kit concept for primary cells. For the 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems we now offer five different Primary Cell Nucleofector™ Solutions P1, P2, P3, P4 and P5.

Each kit contains

- Specific Nucleofector™ Solution
- Supplement
- pmaxGFP™ Control Vector
- Either single 100 µL Nucleocuvettes™, 16-well Nucleocuvette™ Strips, 96-well or 384-well Nucleocuvette™ Plates

All kits are available in different package variations. Please refer to ordering information for details. Optimized Protocols are available for download on our website. In these Optimized Protocols the best Nucleofection Conditions are indicated. In addition, we share our experience and knowledge for treatment of individual primary cell types. You can always find the most up-to-date information in our online cell database.

Benefits

- Five different Nucleofector™ Solutions – One Nucleofector™ Kit can be used for multiple primary cell types
- Conditions are transferable between 4D Nucleofector™ System, 96-well Shuttle™ System and 384-well Nucleofector™ System
- Primary cells maintain functionality post transfection

Applications

- Transfection of lower cell numbers (from 2×10^4 to 1×10^6 cells) and higher cell numbers (from 2×10^5 to 2×10^7 cells) is possible
- Flexible throughput from single cuvette (100 µL) to 16-well Nucleocuvette™ Strip (20 µL), 96-well and 384-well Nucleocuvette™ Plates is possible

www.lonza.com/celldatabase

www.lonza.com/protocols

100 µL Nucleocuvette™ (4D-Nucleofector™ System)	16-well Nucleocuvette™ Strip (4D-Nucleofector™ System)	96-well Nucleocuvette™ Plate (96-well Shuttle™ System)	384-well Nucleocuvette™ Plate (384-well Nucleofector™ System)

Ordering information on the next page.

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Primary Cell Kits for 4D-Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

Continued

Ordering Information – Kits

Cat. No.	Description	Size
4D-Nucleofector™ Kits		
V4XP-1012	P1 Primary Cell 4D-Nucleofector™ X Kit L	12 rxn (100 µL Nucleocuvette™)
V4XP-1024		24 rxn (100 µL Nucleocuvette™)
V4XP-1032	P1 Primary Cell 4D-Nucleofector™ X Kit S	32 rxn (20 µL Nucleocuvette™; 16-well)
V4XP-2012	P2 Primary Cell 4D-Nucleofector™ X Kit L	12 rxn (100 µL Nucleocuvette™)
V4XP-2024		24 rxn (100 µL Nucleocuvette™)
V4XP-2032	P2 Primary Cell 4D-Nucleofector™ X Kit S	32 rxn (20 µL Nucleocuvette™; 16-well)
V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	12 rxn (100 µL Nucleocuvette™)
V4XP-3024		24 rxn (100 µL Nucleocuvette™)
V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	32 rxn (20 µL Nucleocuvette™; 16-well)
V4XP-4012	P4 Primary Cell 4D-Nucleofector™ X Kit L	12 rxn (100 µL Nucleocuvette™)
V4XP-4024		24 rxn (100 µL Nucleocuvette™)
V4XP-4032	P4 Primary Cell 4D-Nucleofector™ X Kit S	32 rxn (20 µL Nucleocuvette™; 16-well)
V4XP-5012	P5 Primary Cell 4D-Nucleofector™ X Kit L	12 rxn (100 µL Nucleocuvette™)
V4XP-5024		24 rxn (100 µL Nucleocuvette™)
V4XP-5032	P5 Primary Cell 4D-Nucleofector™ X Kit S	32 rxn (20 µL Nucleocuvette™; 16-well)
96-well Shuttle™ Kits		
V4SP-1096	P1 Primary Cell 96-well-Nucleofector™ Kit	96 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-1960		960 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-2096	P2 Primary Cell 96-well-Nucleofector™ Kit	96 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-2960		960 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-3096	P3 Primary Cell 96-well-Nucleofector™ Kit	96 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-3960		960 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-4096	P4 Primary Cell 96-well-Nucleofector™ Kit	96 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-4960		960 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-5096	P5 Primary Cell 96-well-Nucleofector™ Kit	96 rxn (20 µL Nucleocuvette™; 96-well)
V4SP-5960		960 rxn (20 µL Nucleocuvette™; 96-well)
384-well Nucleofector™ Kits		
V5SP-1002	P1 Primary Cell 384-well Nucleofector™ Kit	768 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-1010		3840 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-2002	P2 Primary Cell 384-well Nucleofector™ Kit	768 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-2010		3840 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	768 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-3010		3840 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-4002	P4 Primary Cell 384-well Nucleofector™ Kit	768 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-4010		3840 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-5002	P5 Primary Cell 384-well Nucleofector™ Kit	768 rxn (20 µL Nucleocuvette™; 384-well)
V5SP-5010		3840 rxn (20 µL Nucleocuvette™; 384-well)

Primary Cell Kits for 4D-Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

Continued

Quick Reference Guide

Cell types	Efficiency	Viable cells	Solution	Kits for 4D-Nucleofector™ (Cat. No.) 100 µL (12 rxn) Cat. No.	100 µL (24 rxn) Cat. No.	20 µL (32 rxn) Cat. No.	Kits for 96-well Shuttle™ (Cat. No.) 20 µL (96 rxn) Cat. No.	20 µL (960 rxn) Cat. No.
Adipocytes								
Pre-adipocytes, human, visceral	37-94%	35-90%	P1	V4XP-1012	V4XP-1024	V4XP-1032	V4SP-1096	V4SP-1960
Pre-adipocytes, human, subcutaneous	51-84%	33-85%	P1	V4XP-1012	V4XP-1024	V4XP-1032	V4SP-1096	V4SP-1960
Pre-adipocytes, human, visceral (Diabetes Type II)	28-65%	64-84%	P1	V4XP-1012	V4XP-1024	V4XP-1032	V4SP-1096	V4SP-1960
Pre-adipocytes, human, subcutaneous (Diabetes Type II)	31-70%	61-95%	P1	V4XP-1012	V4XP-1024	V4XP-1032	V4SP-1096	V4SP-1960
Bone/Cartilage Cells								
Chondrocyte, human	74%	84%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Dermal Cells								
Keratinocyte, human, neonatal (NHEK)	60–70%	50–60%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Endothelial Cells								
Endothelial, aortic (HAEC), human	73%	70%	P5	V4XP-5012	V4XP-5024	V4XP-5032	V4SP-5096	V4SP-5960
Endothelial, microvascular, lung (HMVEC-L), human	79%	48%	P5	V4XP-5012	V4XP-5024	V4XP-5032	V4SP-5096	V4SP-5960
Endothelial, umbilical vn.(HUVEC), human	90%	55%	P5	V4XP-5012	V4XP-5024	V4XP-5032	V4SP-5096	V4SP-5960
Epithelial Cells								
Epithelial, bronchial (NHBE), human	54%	53%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Epithelial, bronchial, human, asthmatic	72%	75%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Epithelial, bronchial, human, COPD	63%	80%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Epithelial, mammary (HMEC), human	51%	66%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Epithelial, prostate (PrEC), human	67%	48%	P1	V4XP-1012	V4XP-1024	V4XP-1032	V4SP-1096	V4SP-1960
Fibroblasts								
Fibroblast, dermal (NHDF), human – adult	92–96%	92–100%	P2	V4XP-2012	V4XP-2024	V4XP-2032	V4SP-2096	V4SP-2960
Fibroblast, dermal (NHDF), human – neo	98%	86-91%	P2	V4XP-2012	V4XP-2024	V4XP-2032	V4SP-2096	V4SP-2960
Fibroblast , embryonic (MEF), mouse	68%	85-90%	P4	V4XP-4012	V4XP-4024	V4XP-4032	V4SP-4096	V4SP-4960
Hematopoietic Cells								
B cell, mouse, stimulated	55–56%	41–87%	P4	V4XP-4012	V4XP-4024	V4XP-4032	V4SP-4096	V4SP-4960
B cell, peripheral blood, $CD19^+$, human	28%	70%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Dendritic cell, human, immature (mRNA)	69%	84%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Dendritic cell, human, mature (mRNA)	32%	97%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Dendritic cell, mouse, mature – BALB/c	32%	85%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Dendritic cell, mouse, immat. – BALB/c	43%	37–49%	P4	V4XP-4012	V4XP-4024	V4XP-4032	V4SP-4096	V4SP-4960
Dendritic cell, mouse, mature – C57BL/6	29%	88%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Dendritic cell, mouse, immat. – C57BL/6	34%	41–58%	P4	V4XP-4012	V4XP-4024	V4XP-4032	V4SP-4096	V4SP-4960

Primary Cell Kits for 4D-Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

Continued

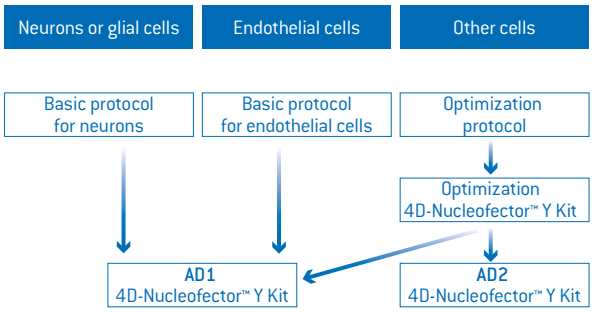
Quick Reference Guide

Cell types	Efficiency	Viable cells	Solution	Kits for 4D-Nucleofector™ (Cat. No.) 100 µL (12 rxn) Cat. No.	100 µL (24 rxn) Cat. No.	20 µL (32 rxn) Cat. No.	Kits for 96-well Shuttle™ (Cat. No.) 20 µL (96 rxn) Cat. No.	20 µL (960 rxn) Cat. No.
Macrophage, human	42%	60%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Macrophage, mouse – BALB/c	33-37%	41%	P2	V4XP-2012	V4XP-2024	V4XP-2032	V4SP-2096	V4SP-2960
Monocyte CD14$^+$, human	64%	77%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
T cell, human stimulated	70%	59%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
T cell, human unstimulated	69–87%	53–79%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
T cell, mouse – BALB/c	45%	32%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
T cell, mouse – C57BL/6	43%	23%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Hepatocytes								
Hepatocyte, human	54%	59–69%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Muscle Cells								
Skeletal Muscle Myoblasts, human	72-78%	61%	P5	V4XP-5012	V4XP-5024	V4XP-5032	V4SP-5096	V4SP-5960
SMC, aortic (AoSMC), human	80%	53–80%	P1	V4XP-1012	V4XP-1024	V4XP-1032	V4SP-1096	V4SP-1960
Neural Cells								
Neuron, cortical, rat	30–50%		P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Neuron, hippocampal, rat	30–50%		P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Stem Cells								
CD34$^+$ cell, bone marrow, human	62-70%	79-91%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
CD34$^+$ cell, cord blood, human	83%	62%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Embryonic stem (ES) cell, human	64%	98%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Embryonic stem (ES) cell, mouse	86–90%	68–81%	P3	V4XP-3012	V4XP-3024	V4XP-3032	V4SP-3096	V4SP-3960
Mesenchymal stem cells (MSC), human	69–78%	67–71%	P1	V4XP-1012	V4XP-1024	V4XP-1032	V4SP-1096	V4SP-1960

Adherent Nucleofector™ Kits for 4D-Nucleofector™ System

For adherent Nucleofection using the 4D-Nucleofector™ Y Unit, specific kits are required including an optimized 24-well Dipping Electrode Array made with conductive polymer electrodes.

Following our new simplified kit strategy invented with the 4D-Nucleofector™ System we offer two Nucleofector™ Solutions called AD1 and AD2, both available as separate kits or combined in to an optimization kit. Each solution may serve different cell types. You can easily find out which solution is optimal for your cell of interest by using the scheme on the right.



Each kit contains

- Specific Nucleofector™ Solution
- Supplement
- pmaxGFP™ Control Vector
- 24-well Dipping Electrode Array
- Nunclon™ Δ Surface 24-well plate (Nunc)

Benefits

- Nucleofection of cells at any time point during this culture period, i.e. at a later developmental stage
- Transfection efficiencies up to 70% combined with high viabilities

Applications

- Two 4D-Nucleofector™ Y Kits that may serve different cell types
- An Optimization 4D-Nucleofector™ Y Kit for primary cells or cell lines lacking an optimized protocol

www.lonza.com/celldatabase

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Adherent Nucleofection Kits				
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
V4YP-2A24	V4YP-2A24	AD2 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
V4YP-9A48	V4YP-9A48	Primary Cell Optimization 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	48 reactions

Related Products	Page
4D-Nucleofector™ Y Unit	175

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Primary Cell Optimization Kits for 4D-Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

The new Primary Cell Optimization Nucleofector™ Kits are the ideal tool to conveniently and rapidly determine Nucleofection Conditions for primary cell types lacking an Optimized Protocol.

Different conditions can easily be tested within one experiment using any of the Nucleofector™ Platforms (4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ System) as all of them are able to address individual wells of a 16-well, 96-well or 384-well Nucleocuvette™ Plate with different programs. In each system our five Primary Cell Nucleofector™ Solutions P1 – P5 are tested together with a pre-selected set of programs plus controls.

Benefits

- Convenient and rapid determination of optimal Nucleofection Conditions for a broad range of primary cells within one experiment
- Optimal Nucleofection Conditions determined on one platform are transferable to the other platforms and also to the 100 µL single Nucleocuvette™ in the 4D-Nucleofector™ X Unit

Applications

- Determination of Nucleofection Conditions for primary cell types lacking an Optimized Protocol

Platform	4D-Nucleofector™ System	96-well Shuttle™ System	384-well Nucleofector™ System
Nucleocuvette™ Vessel			
Kit contents	- Six 16-well Nucleocuvette™ Strips - Specific Nucleofector™ Solution - Supplement - pmaxGFP™ Control Vector	- Two 96-well Nucleocuvette™ Plates - Specific Nucleofector™ Solution - Supplement - pmaxGFP™ Control Vector	- One 384-well Nucleocuvette™ Plate - Specific Nucleofector™ Solution - Supplement - pmaxGFP™ Control Vector
Number of optimization reactions	80 rxn (plus 16 rxn for optional fine tuning)	160 reactions	384 reactions

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)

Primary Cell Kits for Nucleofector™ II/2b Device

The Nucleofector™ II/2b uses cell type specific kits, each of them dedicated to an individual primary cell. Individually developed Nucleofector™ Kits are available for each primary cell type to use in combination with the Nucleofector™ II/2b Device.

Each Kit Contains

- Specific Nucleofector™ Solution
- Supplement
- Single use pipettes
- pmaxGFP™ Control Vector
- Certified 100 µL aluminum electrode cuvettes

All Primary Cell Kits for the Nucleofector™ II/2b Device are available in different package variations (10, 25 and 4 × 25 reactions) and include a CD containing all cell-type specific Optimized Protocols. The best Nucleofection Conditions are indicated in these optimized protocols. In addition we share our experience and knowledge for treatment of individual primary cell types. You can always find the most up-to-date information in our online cell database.

www.lonza.com/celldatabase

See pages 188-241

Quick Reference Guide

Cell types	Efficiency	Viable cells	Kits for Nucleofector™ II/ 2b (Cat. No.) 10 rxn Cat. No.	25 rxn Cat. No.	100 rxn Cat. No.
Bone/Cartilage Cells					
Chondrocyte, human	65%	60-70%		VPF-1001	VVPF-1001
Cardiac Cells					
Cardiomyocyte, rat	75-80%	50-60%	VAPE-1002	VPE-1002	VVPE-1002
Dermal Cells					
Keratinocyte, adult (NHEK-Ad), human	51%	40-60%	VAPD-1002	VPD-1002	VVPD-1002
Keratinocyte, neonatal (NHEK-neo), human	39-53%	50-60%	VAPD-1002	VPD-1002	VVPD-1002
Melanocyte, neonatal (NHEM-neo), human	70%	55-60%	VAPD-1003	VPD-1003	VVPD-1003
Endothelial Cells					
Endothelial, coronary artery (HCAEC), human	57%	42%		VPB-1001	VVPB-1001
Endothelial, microvascular, lung (HMVEC-L), human	52%	52%		VPB-1003	VVPB-1003
Endothelial, umbilical vein (HUVEC), human	90%	60-74%	VAPB-1002	VPB-1002	VVPB-1002
Epithelial Cells					
Epithelial, bronchial (NHBE), human	50-65%	50%			
Epithelial, mammary (HMEC), human	73%	66-98%		VPK-1002	VVPK-1002
Epithelial, prostate (PrEC), human	43%	64%	VAPI-1005	VPI-1005	VVPI-1005
Fibroblasts					
Embryonic fibroblast (MEF), mouse	43%	60-80%	VPD-1006*		
Fibroblast, dermal (NHDF), human – adult	42-69%	74-77%	VAPD-1001	VPD-1001	VVPD-1001
Fibroblast, dermal (NHDF), human – neo	90%	85-90%	VAPD-1001	VPD-1001	VVPD-1001
Hematopoietic Cells					
B cell, peripheral blood, CD19+, human	36%	84-92%	VAPA-1001	VPA-1001	VVPA-1001
B cell, mouse, stimulated	59%	27-47%	VAPA-1010	VPA-1010	VVPA-1010
Dendritic cell, human	93-99%	12-75%	VAPA-1004	VPA-1004	VVPA-1004
Dendritic cell, mouse, immature – BALB/c	58%	62%	VAPA-1011	VPA-1011	VVPA-1011

*Starter Kit: different reaction size.

Primary Cell Kits for Nucleofector™ II/2b Device

Continued

Quick Reference Guide

Cell types	Efficiency	Viable cells	Kits for Nucleofector™ II/ 2b (Cat. No.) 10 rxn Cat. No.	25 rxn Cat. No.	100 rxn Cat. No.
Dendritic cell, mouse, immature – C57BL/6	54%	52%	VAPA-1011	VPA-1011	VVPA-1011
Dendritic cell, mouse, mature – BALB/c	49%	78%	VAPA-1011	VPA-1011	VVPA-1011
Dendritic cell, mouse, mature – C57BL/6	37%	63%	VAPA-1011	VPA-1011	VVPA-1011
Macrophage, human	55-59%	87-88%	VAPA-1008	VPA-1008	VVPA-1008
Macrophage, mouse – BALB/c	34-45%	84-92%	VAPA-1009	VPA-1009	VVPA-1009
Macrophage, mouse – C57BL/6	24-47%	80-88%	VAPA-1009	VPA-1009	VVPA-1009
Monocyte CD14$^+$, human	60%	62-81%		VPA-1007	VVPA-1007
Natural killer (NK), human	54%	50-60%	VAPA-1005	VPA-1005	VVPA-1005
T cell, human stimulated	41-47%	83-90%	VAPA-1002	VPA-1002	VVPA-1002
T cell, human unstimulated	70-75%	85%	VAPA-1002	VPA-1002	VVPA-1002
T cell, mouse – BALB/c	44%	18-55%		VPA-1006	VVPA-1006
T cell, mouse – C57BL/6	20-28%	17-45%		VPA-1006	VVPA-1006
Hepatocytes					
Hepatocyte, mouse	54%	80%	VAPL-1002	VPL-1002	VVPL-1002
Hepatocyte, rat	52%	78%	VAPL-1003	VPL-1003	VVPL-1003
Neural Cells					
Astrocyte, mixed brain, C57 mouse	60%	60-70%	VAPI-1006	VPI-1006	VVPI-1006
Astrocyte, mixed brain, CD1 mouse	60%	60-70%	VAPI-1006	VPI-1006	VVPI-1006
Astrocytes, striatum,rat	67%	70-80%	VAPI-1006	VPI-1006	VVPI-1006
Dorsal root ganglia (DRG), rat	41%		VAPG-1003	VPG-1003	VVPG-1003
Dorsal root ganglia (DRG), chicken	30%			VPG-1002	VVPG-1002
Neuron, cortical, rat	58-67%	47-60%	VAPG-1003	VPG-1003	VVPG-1003
Neuron, hippocampal, chicken	43%			VPG-1002	VVPG-1002
Neuron, hippocampal, rat	58-67%	47-60%	VAPG-1003	VPG-1003	VVPG-1003
Neuron, hippocampal, mouse	58%		VAPG-1001	VPG-1001	VVPG-1001
Oligodendrocyte, rat	44%	60%	VAPI-1006	VPI-1006	VVPI-1006
Smooth Muscle Cells					
Smooth muscle cell, aortic (AoSMC), human	75%	69-96%	VAPC-1001	VPC-1001	VVPC-1001
Stem Cells					
CD34$^+$ cell, bone marrow, human	82%	70%	VAPA-1003	VPA-1003	VVPA-1003
Embryonic stem (ES) cell, human	20-78%	50-96%	VPH-5002*		
Embryonic stem (ES) cell, mouse	87-90%	90-99%	VAPH-1001	VPH-1001	VVPH-1001
Mesenchymal stem cell (MSC), human	55-88%	50-86%	VAPE-1001	VPE-1001	VVPE-1001
Neural stem cell (NSC), mouse	82%		VAPG-1004	VPG-1004	VVPG-1004
Neural stem cell (NSC), rat	42-46%			VPG-1005	VVPG-1005

*Starter Kit: different reaction size

Nucleofector™ Kits for Human Pre-Adipocytes

Optimal kits for transfection of human pre-adipocytes in the 4D-Nucleofector™ X Unit are the P1 Primary Cell Kits, used in combination with the cell-type specific protocol. Due to transferability between all platforms, same conditions apply for the 96-well Shuttle™ or 384-well Nucleofector™ Systems.

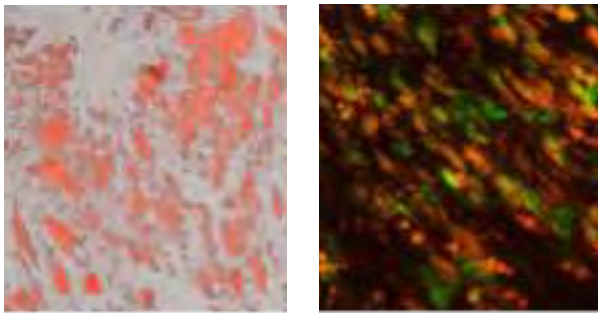
Benefits

- Transfection efficiency: up to 90%
- Viability: up to 80%
- Cells can be differentiated into adipocytes post Nucleofection

Applications

- Validated to work with visceral and subcutaneous Poietics™ Human Preadipocytes
- Also tested with Diabetes Type II pre-adipocytes
- Easily verify previous cell line results in the analogous primary cell type

A B



Example of Nucleofection of human pre-adipocytes. Poietics™ Human Visceral Preadipocytes were transfected with pmaxGFP™ Vector and differentiated into adipocytes post Nucleofection using PGM™ 2 Adipocyte Differentiation Medium. After 14 days cells were analyzed by AdipoRed™ Assay (A) Non-transfected control; (B) transfected cells). Quantitative analysis showed that more than 80% of transfected sample stained positive for AdipoRed (normalized to non-transfected control set to 100%).

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-1012	V4XP-1012	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-1024	V4XP-1024	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-1032	V4XP-1032	P1 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-1960	V4SP-1960	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
V4SP-1096	V4SP-1096	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-1010	V5SP-1010	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
V5SP-1002	V5SP-1002	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)

Related Products	Page
Human Preadipocyte Cells, normal or diseased	27
PGM™ 2 Preadipocyte Growth Medium-2 BulletKit™	28
AdipoRed™ Assay Reagent	271

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Nucleofector™ Kits for Human B Cells

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human B cells using the different Nucleofection Platforms.

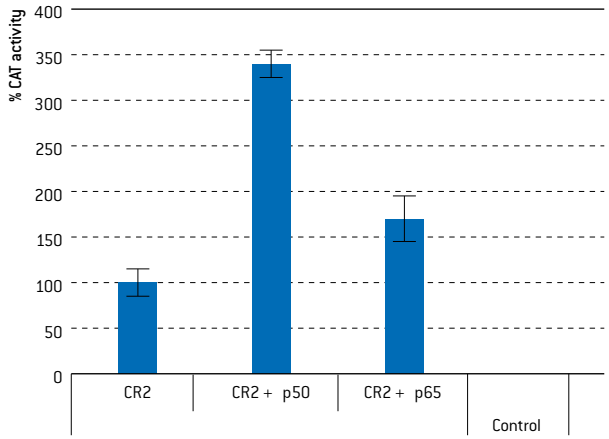
Optimal kits for transfection of human B cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Human B cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 36% using a non-viral method
- Viability: up to 92%

Applications

- Kits suitable for human CD19⁺ B cells from peripheral blood
- Also applicable for transfection of CLL cells derived from patient material
- For both unstimulated and stimulated B cells
- Same conditions for DNA, siRNA, or RNA



Promoter studies in primary B cells prove that NF-KB regulates the activity of the human CR2 promoter. Primary human B cells were transiently co-transfected with a CAT reporter plasmid driven by wild-type (WT) CR2 promoter, and plasmids encoding NF-KB subunit p50 or p65 or a control plasmid. Cells were assayed for CAT activity 15 hours post Nucleofection. Values represent percentage of CAT activity, considering the activity of the empty vector control 0% and the activity of the wild-type CR2 promoter 100%. The results demonstrate that both NF-KB subunits clearly induce CAT activity. (Data reproduced from Tolnay et al. (2002) J Immunology, with permission from the Journal of Immunology.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1001	VAPA-1001	Human B Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1001	VPA-1001	Human B Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1001	VVPA-1001	Human B Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
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LGM™ 3 Lymphocyte Growth Medium-3	102-104
X-VIVO™ 20 Chemically Defined, Serum-free Hematopoietic Cell Medium	126

Nucleofector™ Kits for Stimulated Mouse B Cells

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of stimulated mouse B cells using the different Nucleofection Platforms. Optimal kits for transfection of mouse B cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P4 Primary Cell Kits used in combination with cell-type specific protocols. Mouse B cell specific kits are available for the Nucleofector™ II/2b Device.

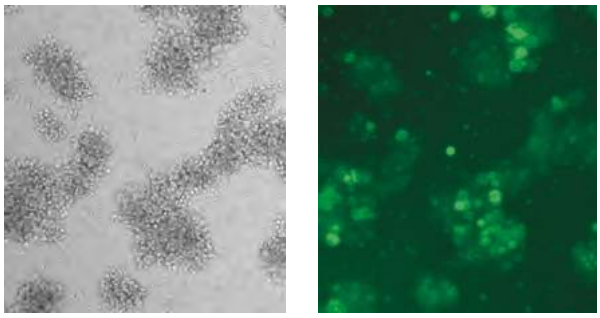
Benefits

- Transfection efficiency: up to 59%
- Viability: up to 87%
- Expression of cell typical marker proteins not affected

Applications

- Kits suitable for stimulated mouse B cells
- Same conditions for DNA, siRNA and RNA transfection

A B



Nucleofection of mouse B cells. Primary mouse B cells were transfected by Nucleofection using a plasmid encoding maxGFP™ Reporter Protein. Cells were then stimulated with LPS. 48 hours post Nucleofection, cells were analyzed by light (A) and fluorescence microscopy (B).

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-4012	V4XP-4012	P4 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-4024	V4XP-4024	P4 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-4032	V4XP-4032	P4 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-4096	V4SP-4096	P4 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-4960	V4SP-4960	P4 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-4002	V5SP-4002	P4 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-4010	V5SP-4010	P4 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1010	VAPA-1010	Mouse B Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1010	VPA-1010	Mouse B Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1010	VVPA-1010	Mouse B Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
RPMI 1640 without L-Glutamine	117

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Nucleofector™ Kits for Human Dendritic Cells

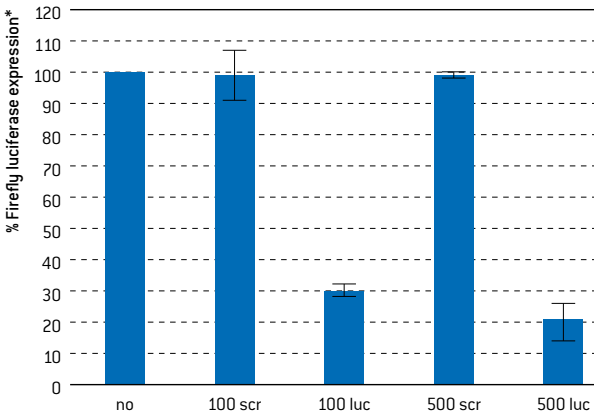
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human dendritic cells using the different Nucleofection Platforms. Optimal kits for transfection of human dendritic cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocolsHuman dendritic cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 49%
- Viability: up to 71%
- Same transfection conditions with different substrates, such as RNA, DNA or siRNA

Applications

- Kit suitable for immature and mature monocyte-derived dendritic cells
- For short term expression of up to 48 hours



Co-transfection of human DCs with plasmid and siRNA. Cells were transfected by Nucleofection with a CMV promoter driven firefly luciferase vector (pCMV-Luc), TK-promoter driven Renilla luciferase vector (pTK-Luc) as internal control reporter for normalization, and siRNA against firefly luciferase (luc) or scrambled control (scr). 24 hours post Nucleofection, cells were analyzed for luciferase activity. (Data reproduced from Stallwood *et al.* (2006) *J Immunol* **177(2)**:885-895, with permission of the authors).

*Normalized to Renilla luciferase as internal control reporter

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1004	VAPA-1004	Human Dendritic Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1004	VPA-1004	Human Dendritic Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1004	VVPA-1004	Human Dendritic Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

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LGM™ 3 Lymphocyte Growth Medium-3	102-104
Normal Human Dendritic Cells	102

Nucleofector™ Kits for Mouse Dendritic Cells

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of mouse dendritic cells (DCs) using the different Nucleofection Platforms.

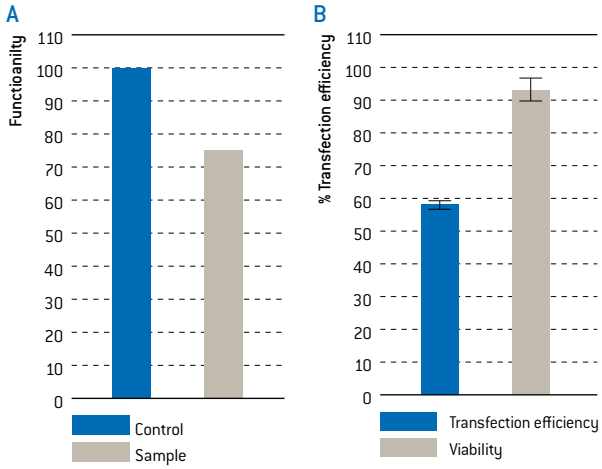
Optimal kits for transfection of mouse dendritic cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits (for mature DCs) and P4 Primary Cell Kits (for immature DCs) used in combination with cell-type specific protocols. Mouse dendritic cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 58% using a non-viral method
- Viability: up to 88%

Applications

- Kits suitable for Balb/C or C57BL/6 mouse DCs
- Proven results for immature and mature mouse DCs
- Ideal for gene over-expression studies or RNAi mediated gene silencing



Transfection efficiency and functionality of mouse DCs post Nucleofection. (A) The graph displays functionality of immature mouse DCs (isolated from mouse strain Balb/C) post Nucleofection (Sample). Two hours post Nucleofection, cells were stimulated by LPS. 22 hours later, functionality was analyzed by IL-6 specific ELISA and is given in percent compared to non-transfected control. (B) Mouse DC (Balb/C) were transfected using pmaxGFP™ Vector. Cells were analyzed 24 hours post Nucleofection by flow cytometry for maxGFP™ Reporter Protein expression and viability. Cell viability is given in percent compared to non-transfected control.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L*	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L*	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S*	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4XP-4012	V4XP-4012	P4 Primary Cell 4D-Nucleofector™ X Kit L**	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-4024	V4XP-4024	P4 Primary Cell 4D-Nucleofector™ X Kit L**	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-4032	V4XP-4032	P4 Primary Cell 4D-Nucleofector™ X Kit S**	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit*	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit*	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
V4SP-4096	V4SP-4096	P4 Primary Cell 96-well Nucleofector™ Kit**	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-4960	V4SP-4960	P4 Primary Cell 96-well Nucleofector™ Kit**	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit*	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit*	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
V5SP-4002	V5SP-4002	P4 Primary Cell 384-well Nucleofector™ Kit**	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-4010	V5SP-4010	P4 Primary Cell 384-well Nucleofector™ Kit**	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1011	VAPA-1011	Mouse Dendritic Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1011	VPA-1011	Mouse Dendritic Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1011	VVPA-1011	Mouse Dendritic Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

*For mature Mouse DCs **For immature Mouse DCs

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Nucleofector™ Kits for Human Macrophages

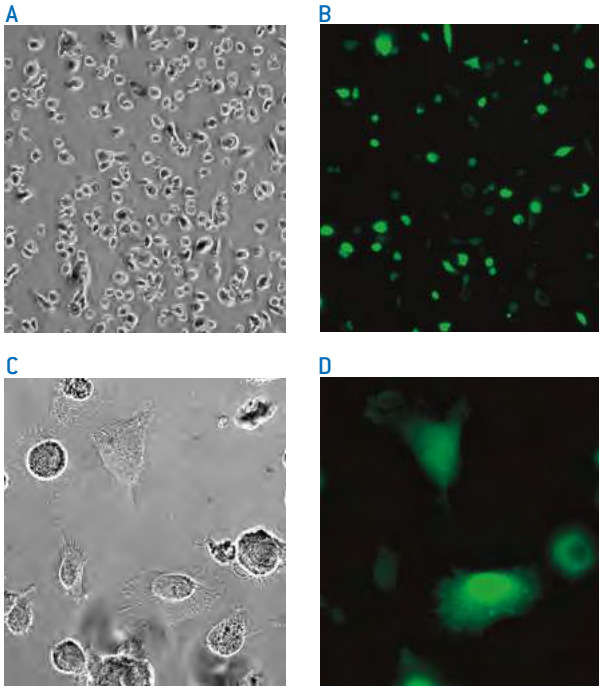
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human macrophages using the different Nucleofection Platforms. Optimal kits for transfection of human macrophages in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Human macrophage specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 59%
- Viability: up to 88%
- Maintenance of functionality (e.g. activation)

Applications

- Kits suitable for resting human macrophages
- Cited for DNA and siRNA transfection
- High-throughput screening approaches possible



Nucleofection of human macrophages. Primary human macrophages were transfected by Nucleofection with pmaxGFP™ Control Vector. 24 hours post Nucleofection, cells were analyzed for maxGFP™ Reporter Protein expression by light (A, C) and fluorescence (B, D) microscopy. A and B show cells at 10x magnification. At 40x magnification (C, D) transfected macrophages reveal cytoplasmatic extrusions important for phagocytic function of macrophages.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1008	VAPA-1008	Human Macrophage Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1008	VPA-1008	Human Macrophage Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1008	VVPA-1008	Human Macrophage Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
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Nucleofector™ Kits for Mouse Macrophages

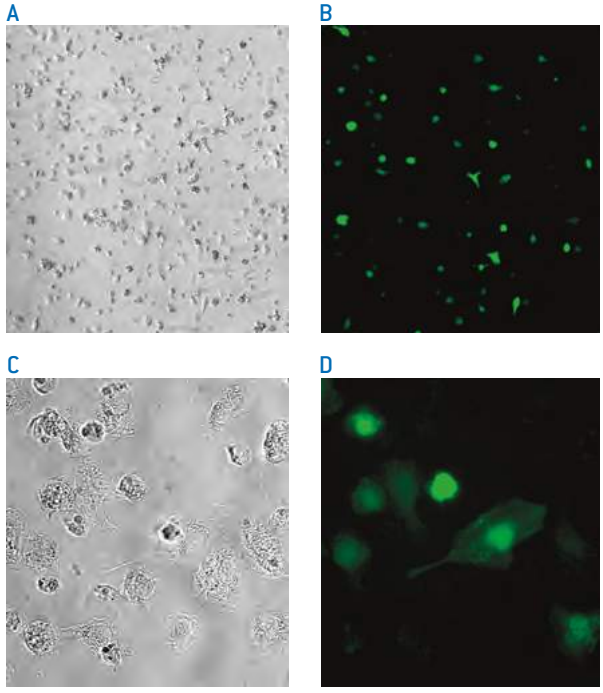
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of mouse macrophages using the different Nucleofection Platforms. Optimal kits for transfection of mouse macrophages in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P2 Primary Cell Kits used in combination with cell-type specific protocols Mouse macrophage specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 47%
- Viability: up to 92%
- Maintenance of functionality (e.g., activation)

Applications

- Kits suitable for resting bone marrow-derived mouse macrophages
- Evaluated for C57BL/6 and BALB/c strains
- Enabling studies of gene regulation, signaling pathways or differentiation



Nucleofection of mouse macrophages with pmaxGFP™ Vector. Primary mouse macrophages were transfected by Nucleofection with a plasmid encoding maxGFP™ Reporter Protein. 24 hours post Nucleofection, cells were analyzed by light (A, C) and fluorescence microscopy (B, D). A and B show cells at 10 × magnification. At 40 × magnification (C, D), transfected macrophages reveal cytoplasmic extrusions important for phagocytic function.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-2012	V4XP-2012	P2 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-2024	V4XP-2024	P2 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-2032	V4XP-2032	P2 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-2096	V4SP-2096	P2 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-2960	V4SP-2960	P2 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-2002	V5SP-2002	P2 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-2010	V5SP-2010	P2 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1009	VAPA-1009	Mouse Macrophage Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1009	VPA-1009	Mouse Macrophage Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1009	VVPA-1009	Mouse Macrophage Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
DMEM 4.5 g/L glucose with L-Glutamine	109
RPMI 1640 without L-Glutamine	117

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Nucleofector™ Kits for Human Monocytes

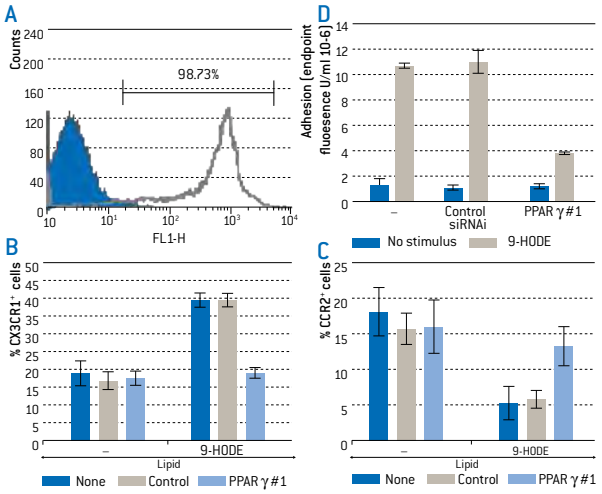
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human monocytes using the different Nucleofection Platforms. Optimal kits for transfection of human monocytes in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Human monocyte specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 64%
- Viability: up to 81%
- First high-throughput transfection technology for human monocytes

Applications

- Kits suitable for CD14⁺ human monocytes
- Cited for DNA and siRNA transfections



Nucleofection™ of human monocytes with Stealth™ siRNA. (A) Efficiency of transfection was determined with 100 nM fluorescein-labeled dsRNA oligomer (same length, electrical charge and configuration as the siRNA) monitored 24 hours later by flow cytometry. Blue curve shows autofluorescence. (B, C and D) Knockdown with Stealth™ siRNA (Invitrogen). Oxidized linoleic acid metabolites (like 9-HODE, 9-hydroxy-10E, 12Z-octadecadienoic acid ester), components of oxidized LDL found in large amounts in atherosclerotic plaque, are able to specifically induce differentiation of human monocytes to macrophages accompanied by a switch of chemokine receptor expression (CCR2-off and CX3CR1-on). CX3CR1 then mediates macrophage adhesion to coronary arter y smooth muscle cells (CASMCs). The effects of the lipids on receptor expression are mediated by the nuclear receptor peroxisome proliferator-activated receptor (PPAR)γ. Down regulation of PPARγ with siRNA (200 nM, (Invitrogen)) dramatically reduced receptor switch (B and C) and consequently macrophage adhesion to CASMCs in an adhesion assay (D). (Data extracted from Barlic et al., (2006) Circulation 114(8), 807-19 with permission from the authors.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VPA-1007	VPA-1007	Human Monocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1007	VVPA-1007	Human Monocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
LGM™ 3 Lymphocyte Growth Medium-3	102-104
Human CD14⁺ Monocytes	103

Nucleofector™ Kits for Human Natural Killer Cells

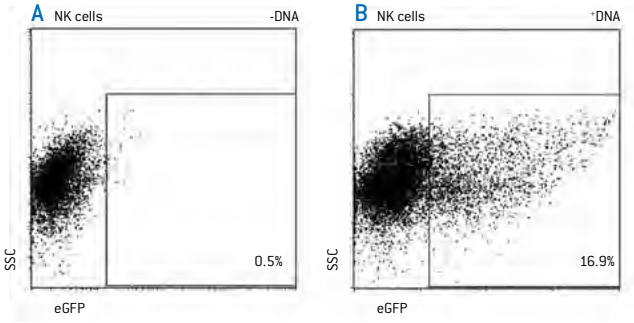
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human NK cells using the different Nucleofection Platforms.
For the transfection of human NK cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System we recommend using the Primary Cell Optimization Kits and the respective optimization protocols. Optimal Nucleofection Conditions are transferable between these three systems. Human NK cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 54%
- Viability: up to 60%
- Efficient non-viral transfection technology for primary NK cells

Applications

- Kits suitable for human $CD56^{+}/CD3^{-}$ natural killer cells



Nucleofection of primary human NK cells. Polyclonal human NK cells generated from PBMC co-cultured with the feeder cell line RPMI 8866 for 9 days were transfected by Nucleofection with a plasmid encoding eGFP protein. Cells were analyzed by flow cytometry 24 hours post Nucleofection. eGFP expression in natural killer cells is shown after Nucleofection without (A) and with plasmid DNA (B). (Courtesy of J. Sundback and K. Karre, Karolinska Institute, Microbiology and Tumor Biology Center, Stockholm, Sweden.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1005	VAPA-1005	Human Natural Killer Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1005	VPA-1005	Human Natural Killer Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1005	VVPA-1005	Human Natural Killer Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
LGM™ 3 Lymphocyte Growth Medium-3	102-104
RPMI 1640 without L-Glutamine	117
Human NK Cells	104

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Nucleofector™ Kits for Human T Cells

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human T cells using the different Nucleofection Platforms.
Optimal kits for transfection of human T cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Human T cell specific kits are available for the Nucleofector™ II/2b Device.

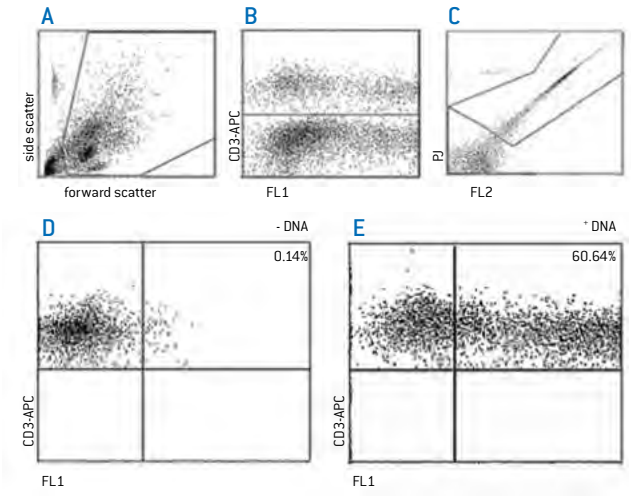
Benefits

- Transfection efficiency: up to 87%
- Viability: up to 90%
- Transfected cells preserve their biochemical functionality
- More than 270 publications on T cell Nucleofection

Applications

- Kits suitable for stimulated and unstimulated human T cells
- RNAi screenings in primary T cells for basic and pharmaceutical research

www.lonza.com/citations



Nucleofection™ of human T cells with pmaxGFP™ Vector. PBMC were freshly isolated from a buffy coat and transfected by Nucleofection with pmaxGFP™ Vector. 24 hours post Nucleofection, cells were analyzed by flow cytometry. Lymphocytes were gated according to forward/side scatter (A). T cells were stained with antibody directed against CD3. Dead cells were excluded by propidium iodide staining and gating (B, C). maxGFP™ Reporter Protein expression of T cells is shown after Nucleofection without (D) and with plasmid DNA (E).

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1002	VAPA-1002	Human T Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1002	VPA-1002	Human T Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1002	VVPA-1002	Human T Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
LGM™ 3 Lymphocyte Growth Medium-3	102-104
IMDM with HEPES and L-Glutamine	113
Human CD4+ T Cells	104

Nucleofector™ Kits for Mouse T Cells

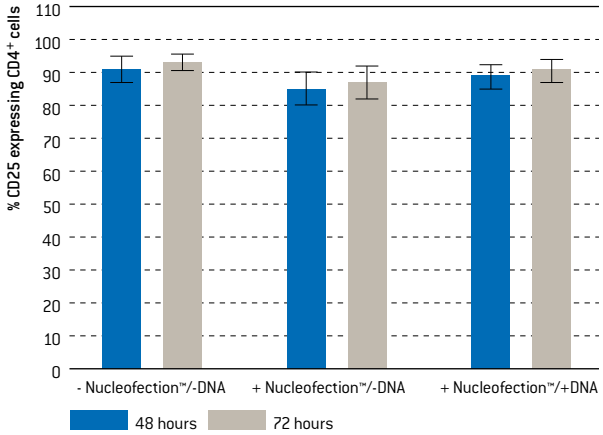
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of mouse T cells using the different Nucleofection Platforms.
Optimal kits for transfection of mouse T cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Mouse T cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 45%
- Viability: up to 55%
- Evaluated for C57BL/6 and BALB/c strains
- Maintenance of functionality, e.g. stimulation

Applications

- Kits suitable for mouse T cells from C57BL/6 or BALB/c
- Overexpression or gene silencing studies possible in high-throughput frameworks



Transfected and non-transfected mouse T cells can be stimulated equally well. Primary C57BL/6 mouse T cells were transfected using Nucleofection with pmaxGFP™ Vector. 3 hours post Nucleofection, cells were stimulated with plate bound anti-CD3 and anti-CD28. 48 and 72 hours post Nucleofection, CD4+ cells were analyzed for CD25 surface expression. Figure shows proportion of CD25-expressing cells among living CD4+ T cells (%CD25 expression in unstimulated samples ranged from 10–20%).

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VPA-1006	VPA-1006	Mouse T Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1006	VVPA-1006	Mouse T Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Mouse T Cell Nucleofector™ Medium	245

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Nucleofector™ Kits for Mammalian Blood Cells

For mammalian blood cells lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection conditions.
The Primary Cell Optimization Kits are suited for optimizations of mammalian blood cells on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.

Benefits

- Protocols guiding through the optimization procedure
- Optimizations can be performed within one experiment
- Optional result fine tuning with help from our Scientific Support Team

Applications

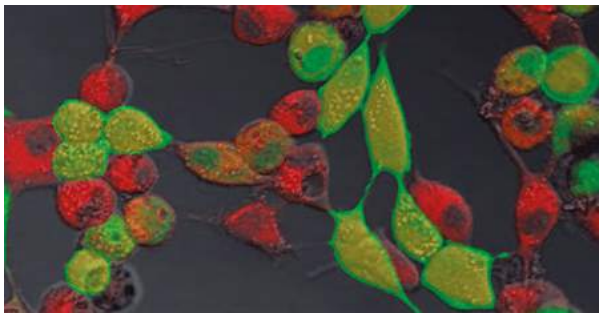
- Kits suited for blood cells from different mammalian species and various organs

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)

Nucleofector™ Kits for Human Chondrocytes

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human chondrocytes using the different Nucleofection Platforms. Optimal kits for transfection of human chondrocytes in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Human chondrocyte cell specific kits are available for the Nucleofector™ II/2b Device.



Example of the transfection of human chondrocytes with eGFP. Human chondrocytes were transfected by Nucleofection using a plasmid encoding the enhanced green fluorescent protein eGFP. Cell membranes were fluorescently stained in red with the substance R18 (Octadecylrhodamine-B-chloride, Molecular Probes). 24 hours post Nucleofection, the cells were analyzed by fluorescence microscopy. The image shows an overlay of eGFP and R18 fluorescence. (Data courtesy of Dr. Schmid and Dr. Aigner, University of Erlangen, Germany.)

Benefits

- Transfection efficiency: up to 74%
- Viability: up to 84%
- First efficient non-viral transfection technology

Applications

- Optimal for studies of degenerative processes, such as osteoarthritis

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VPF-1001	VPF-1001	Human Chondrocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPF-1001	VVPF-1001	Human Chondrocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Human Chondrocytes	84
CGM™ Chondrocyte Growth Medium	85
CGM™ Chondrocyte Differentiation Medium	85

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Nucleofector™ Kits for Rat Cardiomyocytes

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of rat cardiomyocytes using the different Nucleofection Platforms.

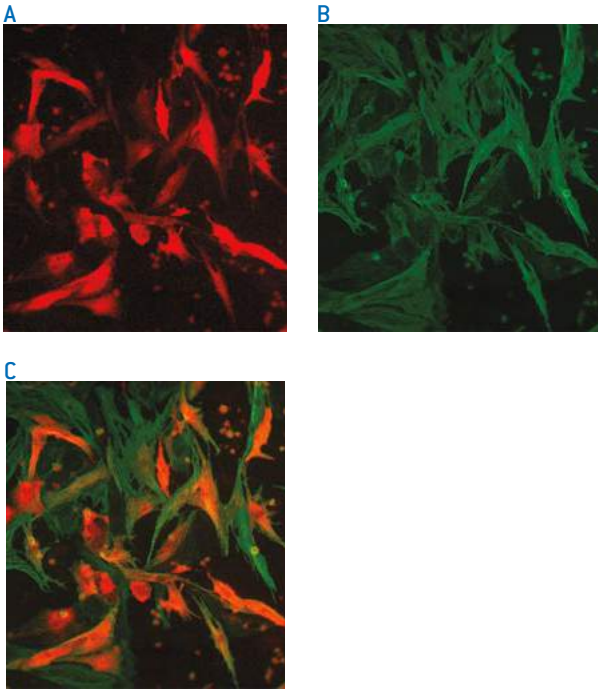
For the transfection of rat cardiomyocytes in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System we recommend using the Primary Cell Optimization Kits and the respective optimization protocols. Optimal Nucleofection Conditions are transferable between these three systems. Rat cardiomyocyte specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 80%
- Viability: up to 60%
- First efficient non-viral transfection technology

Applications

- Kit suitable for neonatal rat cardiomyocytes
- Optimal for studies of cardiac gene regulation and differentiation



Example for Nucleofection of neonatal rat cardiomyocytes with DsRed2 cDNA. Primary neonatal rat cardiomyocytes were transfected by Nucleofection using a plasmid encoding DsRed (Clontech). 2 days post Nucleofection, the cells were analyzed by fluorescence microscopy. Fig. (A) shows DsRed expressing cells. Cardiomyocytes stained with FITC-labeled tropomyosin antibody are shown in Fig. (B). Fig. (C) is an overlay of images (A) and (B). (Photograph courtesy of F. Engel and M. Keating, Cardiology Department, Children's Hospital, Havard Medical School, Boston, Massachusetts, USA.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPE-1002	VAPE-1002	Rat Cardiomyocyte - Neonatal Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPE-1002	VPE-1002	Rat Cardiomyocyte - Neonatal Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPE-1002	VVPE-1002	Rat Cardiomyocyte - Neonatal Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Rat Cardiac Myocytes	92
RCGM – Rat Cardiac Growth Myocytes BulletKit™	92

Nucleofector™ Kits for Human Keratinocytes (NHEK)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human keratinocytes using the different Nucleofection Platforms. Optimal kits for transfection of human keratinocytes in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Human keratinocyte specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 53%
- Viability: up to 60%
- Maintenance of functionality, e.g. no terminal differentiation

Applications

- Validated to work with Clonetics™ Human Keratinocytes
- Kits suitable for adult and neonatal keratinocytes
- Optimal for studying gene expression or intracellular signaling
- Cited for DNA and siRNA transfections

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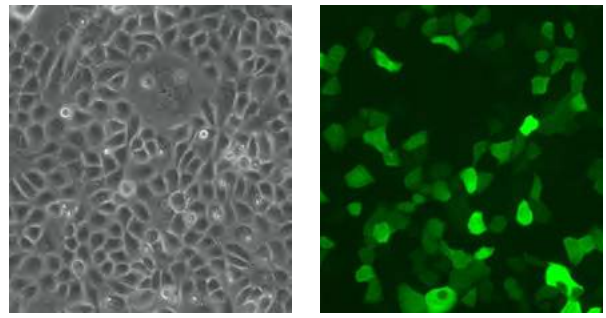

Example for the Nucleofection of human keratinocytes. Clonetics™ NHEK-neo were transfected by Nucleofection with pmaxGFP™ Vector. 48 hours post Nucleofection, cells were analyzed by light (A) and fluorescence microscopy (B).

www.lonza.com/citations

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPD-1002	VAPD-1002	Human Keratinocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPD-1002	VPD-1002	Human Keratinocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPD-1002	VVPD-1002	Human Keratinocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
NHEK – Adult Normal Human Epidermal Keratinocytes	61
NHEK – Neonatal Normal Human Epidermal Keratinocytes	61
KGM™ Gold Keratinocyte Growth Medium BulletKit™	62

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Nucleofector™ Kits for Human Melanocytes (NHEM-Neo)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human melanocytes using the different Nucleofection Platforms.
For the transfection of human melanocytes in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System we recommend using the Primary Cell Optimization Kits and the respective optimization protocols. Optimal Nucleofection Conditions are transferable between these three systems. Human melanocyte specific kits are available for the Nucleofector™ II/2b Device.

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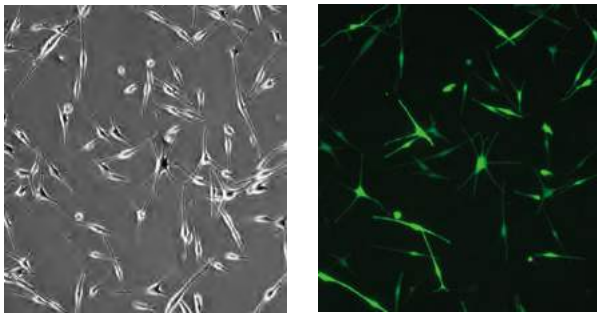


Nucleofection of NHEM-Neo with eGFP cDNA. NHEM-Neo were transfected by Nucleofection using a plasmid encoding enhanced green fluorescent protein, eGFP. 24 hours post Nucleofection, cells were analyzed by light (A) and fluorescence microscopy (B).

Benefits

- Transfection efficiency: up to 70%
- Viability: up to 60%
- Reproducible non-viral transfection

Applications

- Kits suitable for neonatal human melanocytes (NHEM-neo)
- Optimal for both DNA and siRNA transfection

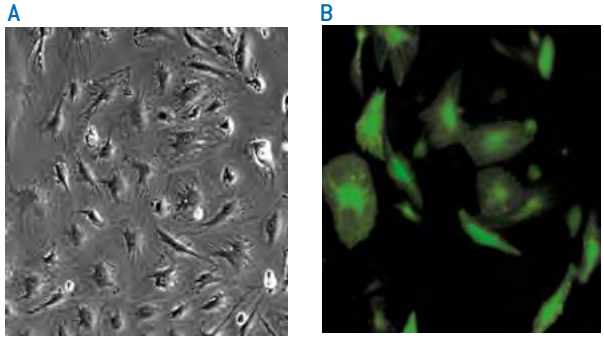
Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPD-1003	VAPD-1003	Human Epidermal Melanocyte - Neonatal Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPD-1003	VPD-1003	Human Melanocyte - Neonatal Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPD-1003	VVPD-1003	Human Epidermal Melanocyte - Neonatal Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
NHEM-Neo – Neonatal Normal Human Epidermal Melanocytes	61
MGM™ 4 Melanocyte Growth Medium-4 BulletKit™	62

Nucleofector™ Kits for Human Coronary Artery Endothelial Cells (HCAEC)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of HCAECs using the different Nucleofection Platforms.
Optimal kits for transfection of HCAECs in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P5 Primary Cell Kits used in combination with respective basic protocols for mammalian endothelial cells. HCAEC specific kits are available for the Nucleofector™ II/2b Device.



Example for the Nucleofection of HCAEC. Clonetics™ HCAEC were transfected by Nucleofection with a plasmid encoding the fluorescent protein eGFP. 25 hours post Nucleofection, cells were analyzed by light (A) and fluorescence microscopy (B).

Benefits

- Transfection efficiency: up to 57%
- Viability: up to 42%

Applications

- Validated to work with Clonetics™ HCAEC
- Ideal for cardiovascular research e.g., on thrombosis, atherosclerosis or hypertension

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-5012	V4XP-5012	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-5024	V4XP-5024	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-5032	V4XP-5032	P5 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-5096	V4SP-5096	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-5960	V4SP-5960	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-5002	V5SP-5002	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-5010	V5SP-5010	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VPB-1001	VPB-1001	Human Coronary Artery Endothelial Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPB-1001	VVPB-1001	Human Coronary Artery Endothelial Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
HCAEC – Human Coronary Artery Endothelial Cells	58,64
D-HCAEC – Diseased Human Coronary Aortic Endothelial Cells (Diabetes Type I or II)	58,64
EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	59, 64

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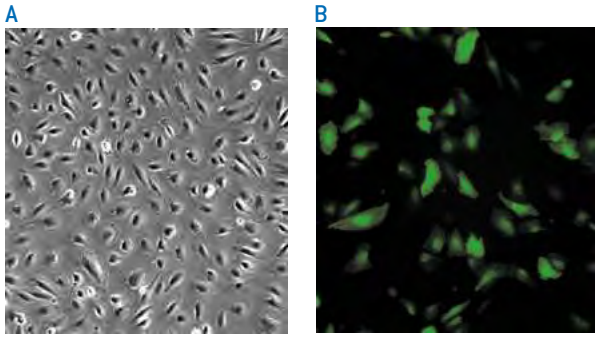
Nucleofector™ Kits for Human Microvascular Endothelial Cells – Lung (HMVEC-L)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of HMVEC-L using the different Nucleofection Platforms.
Optimal kits for transfection of HMVEC-L in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P5 Primary Cell Kits used in combination with respective basic protocols for mammalian endothelial cells. HMVEC-L specific kits are available for the Nucleofector™ II/2b Device.



Example for the Nucleofection of Clonetics™ HMVEC-L. HMVEC-L were transfected by Nucleofection using a plasmid encoding the enhanced green fluorescent protein eGFP. 25 hours post Nucleofection, cells were analyzed by light (A) and fluorescence microscopy (B).

Benefits

- Transfection efficiency: up to 79%
- Viability: up to 52%
- Efficient transfection of HMVEC-L without the use of a viral system

Applications

- Validated to work with Clonetics™ HMVEC-L

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-5012	V4XP-5012	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-5024	V4XP-5024	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-5032	V4XP-5032	P5 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-5096	V4SP-5096	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-5960	V4SP-5960	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-5002	V5SP-5002	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-5010	V5SP-5010	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VPB-1003	VPB-1003	Human Microvascular Endothelial Cell-Lung Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPB-1003	VVPB-1003	Human Microvascular Endothelial Cell-Lung Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
HMVEC-L – Human Microvascular Endothelial Cells – Lung	66, 78
EGM™ 2MV Microvascular Endothelial Cell Growth Medium-2 BulletKit™	67, 79

Nucleofector™ Kits for Human Umbilical Vein Endothelial Cells (HUVEC)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of HUVECs using the different Nucleofection Platforms.
Optimal kits for transfection of HUVECs in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P5 Primary Cell Kits used in combination with cell-type specific protocols. HUVEC specific kits are available for the Nucleofector™ II/2b Device.

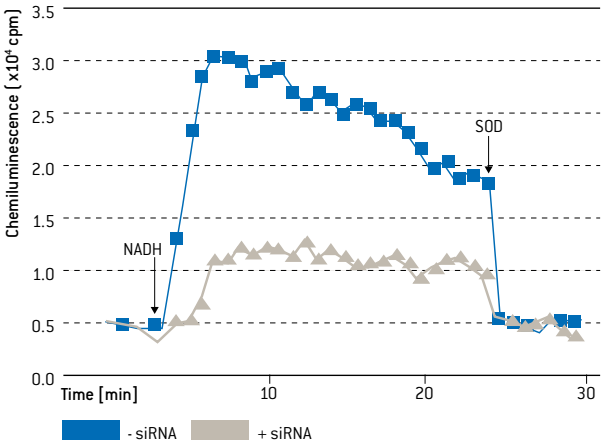
Benefits

- Transfection efficiency: up to 90%
- Viability: up to 74%
- High protein expression levels possible
- More than 60 publications on HUVEC Nucleofection

Applications

- Validated to work with Clonetics™ HUVEC
- Ideal for siRNA screening in drug discovery projects

New Nucleofection in adherent state is possible using the AD1 4D-Nucleofector™ Y Kit



Nucleofection of HUVECs with siRNA. Knockdown of the NAD(P)H oxidase Nox4 with siRNA shows that Nox4 is the major source for superoxide production in the nucleus of HUVECs. 48 hours after Nucleofection of HUVECs with Nox4 siRNA, the nuclear fraction was prepared and superoxide production was determined as superoxide dismutase (SOD)-inhibitable chemiluminescence detected with a luminol-based test. The reaction was started by the addition of NADH and stopped by addition of SOD. (Data from Kuroda et al. (2005) Genes Cells 10(12), 1139-1151.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-5012	V4XP-5012	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-5024	V4XP-5024	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-5032	V4XP-5032	P5 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
96-well Shuttle™ Kits				
V4SP-5096	V4SP-5096	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-5960	V4SP-5960	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-5002	V5SP-5002	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-5010	V5SP-5010	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPB-1002	VAPB-1002	Human Umbilical Vein Endothelial Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPB-1002	VPB-1002	Human Umbilical Vein Endothelial Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPB-1002	VVPB-1002	Human Umbilical Vein Endothelial Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
HUVEC – Human Umbilical Vein Endothelial Cells	64
EGM™ 2 Endothelial Cell Growth Medium-2 BulletKit™	64

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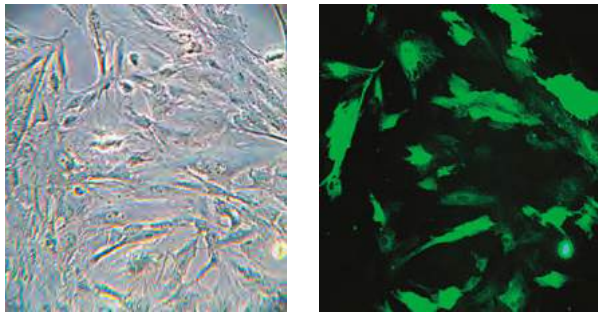
Nucleofector™ Kits for Mammalian Endothelial Cells

For mammalian endothelial cells lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection Conditions.
The P5 Primary Cell Kits together with the cell group-specific Basic Protocols are suited for optimizations of mammalian endothelial cells on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.
A cell-group specific Basic Kit is suited for optimization of mammalian endothelial cells using the Nucleofector™ II/2b Device.

Benefits

- Optimizations can be performed within one experiment
- Detailed protocols provide guidance through the optimization procedure
- Fine tuning of results is possible with the help of our Scientific Support Team
- Transfection efficiency: up to 90%
- Viability: up to 85%

A B



Example for Nucleofection of primary porcine endothelial cells. Primary porcine trabecular meshwork cells (derived from eye) were transfected by Nucleofection with a plasmid encoding the green fluorescent maxGFP™ Reporter Protein. 24 hours post Nucleofection, the cells were analyzed by light (A) and fluorescence microscopy (B). (Data courtesy of Dr. Ted Acott, Oregon Health & Science University, USA.)

Applications

- Kits suited for endothelial cells from different mammalian species and various organs
- Already tested for human pulmonary artery endothelial cells (Clonetics™ HPAEC), porcine capillary endothelial cells, sheep uterine artery endothelial cells, etc
- New Nucleofection in adherent state is possible using the AD1 4D-Nucleofector™ Y Kit

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-5012	V4XP-5012	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-5024	V4XP-5024	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-5032	V4XP-5032	P5 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
96-well Shuttle™ Kits				
V4SP-5096	V4SP-5096	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-5960	V4SP-5960	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-5002	V5SP-5002	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-5010	V5SP-5010	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPI-1001	VAPI-1001	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Endothelial Cells	100 µL aluminum cuvette	10 reactions
VPI-1001	VPI-1001	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Endothelial Cells	100 µL aluminum cuvette	25 reactions
VVPI-1001	VVPI-1001	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Endothelial Cells	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Endothelial Cells and Media	63-67

Nucleofector™ Kits for Human Bronchial Epithelial Cells (NHBE)

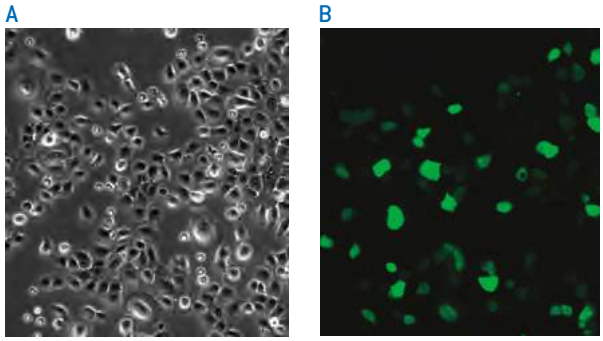
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of NHBEs using the different Nucleofection Platforms.
Optimal kits for transfection of NHBEs in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. NHBE specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 65%
- Viability: up to 53%

Applications

- Validated to work with Clonetics™ NHBE
- Also tested with asthmatic and COPD bronchial epithelial cells
- Easily verify previous cell line results in the analogous primary cell type



Example of Nucleofection of NHBE. Clonetics™ Normal Human Bronchial Epithelial Cells were transfected with pmaxGFP™ Vector. 24 hours post Nucleofection, cells were analyzed by light (A) or fluorescence (B) microscopy.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	**V4XP-3012**	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	**V4XP-3024**	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	**V4XP-3032**	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	**V4SP-3096**	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	**V4SP-3960**	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	**V5SP-3002**	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	**V5SP-3010**	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPI-1005	VAPI-1005	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Epithelial Cells	100 µL aluminum cuvette	10 reactions
VPI-1005	VPI-1005	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Epithelial Cells	100 µL aluminum cuvette	25 reactions
VVPI-1005	**VVPI-1005**	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Epithelial Cells	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
NHBE – Bronchial /Tracheal Epithelial Cells	78
BEGM™ – Bronchial Epithelial Growth Medium BulletKit™	78
DHBE Diseased Bronchial/Tracheal Epithelial Cells (Asthma, COPD, or Cystic Fibrosis)	77-78

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Nucleofector™ Kits for Human Mammary Epithelial Cells (HMEC)

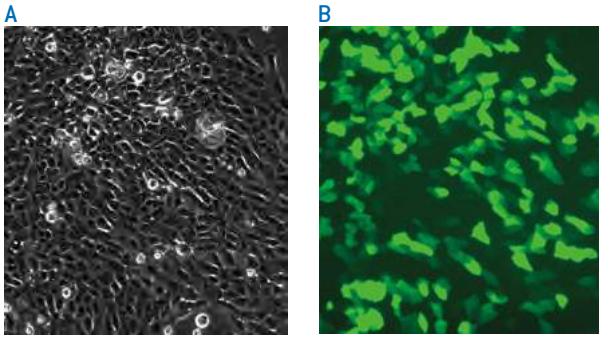
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of HMECs using the different Nucleofection Platforms.
Optimal kits for transfection of HMECs in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. HMEC specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 73%
- Viability: up to 95%

Applications

- Validated to work with Clonetics™ HMEC
- Easily verify previous cell line results in the analogous primary cell type



Example of Nucleofection of HMEC. Clonetics™ Human Mammary Epithelial Cells were transfected with pmaxGFP™ Vector. 24 hours post Nucleofection, cells were analyzed by light (A) or fluorescence (B) microscopy.

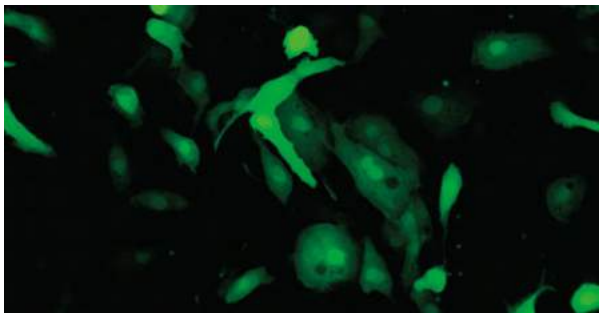
Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VPK-1002	VPK-1002	Human Mammary Epithelial Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPK-1002	VVPK-1002	Human Mammary Epithelial Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
HMEC – Human Mammary Epithelial Cells	70
MEGM™ – Mammary Epithelial Cell Growth Medium BulletKit™	70

Nucleofector™ Kits for Mammalian Epithelial Cells

For mammalian epithelial cells lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection Conditions.
The P1 and P3 Primary Cell Kits together with the cell-group specific Basic Protocols are suited for optimizations of mammalian epithelial cells on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.
A cell group-specific Basic Kit is suited for optimization of mammalian epithelial cells using the Nucleofector™ II/2b Device.



Example for Nucleofection of primary renal proximal tubular epithelial cells. Human renal proximal tubular epithelial cells were transfected by Nucleofection with a plasmid encoding the green fluorescent protein, eGFP. 48 hours post Nucleofection, cells were analyzed by fluorescence microscopy. (Data courtesy of C. Xu, R. L. Bacallao*, and S. L. Alper. Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA, *University of Indiana School of Medicine, Indianapolis, USA.)

Benefits

- Optimizations can be performed within one experiment
- Detailed protocols provide guidance through the optimization procedure
- Fine tuning of results is possible with help from our Scientific Support Team
- Transfection efficiency: up to 83%
- Viability: up to 98%

Aplications

- Kits suited for epithelial cells from different mammalian species and various organs
- Already tested for renal proximal tubular epithelial cells (RPTEC), Clonetics™ Epithelial Cells: human prostate epithelial cells (hPrEC) and human small airway epithelial cells (SAEC)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-1012	V4XP-1012	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-1024	V4XP-1024	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-1032	V4XP-1032	P1 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-1096	V4SP-1096	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-1960	V4SP-1960	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-1002	V5SP-1002	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-1010	V5SP-1010	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPI-1005	VAPI-1005	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Epithelial Cells	100 µL aluminum cuvette	10 reactions
VPI-1005	VPI-1005	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Epithelial Cells	100 µL aluminum cuvette	25 reactions
VVPI-1005	VVPI-1005	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Epithelial Cells	100 µL aluminum cuvette	4 × 25 reactions

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Nucleofector™ Kits for Human Dermal Fibroblasts (NHDF)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of NHDF cells using the different Nucleofection Platforms.
Optimal kits for transfection of NHDF cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P2 Primary Cell Kits used in combination with cell-type specific protocols. NHDF cell specific kits are available for the Nucleofector™ II/2b Device.

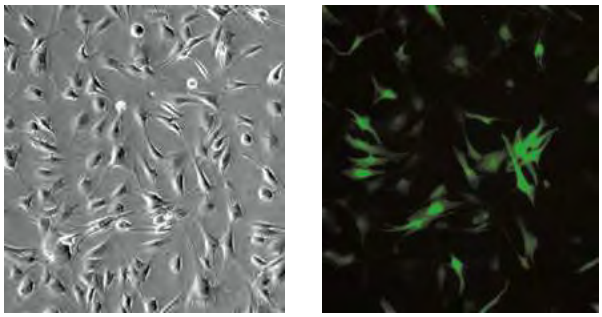
Benefits

- Transfection efficiency: up to 90%
- Viability: up to 98%
- More than 90 publications citing Nucleofection of human dermal fibroblasts

Applications

- Validated to work with Clonetics™ NHDF, neonatal and adult
- Ideal for studying fibrosarcoma, fibrosis, scleroderma, or xeroderma pigmentosum
- Optimal for both DNA and siRNA transfection

A B



Nucleofection of adult human dermal fibroblasts with eGFP cDNA. Clonetics™ NHDF-Adult were transfected by Nucleofection using a plasmid encoding eGFP. 24 hours post Nucleofection, cells were analyzed by light (A) and fluorescence microscopy (B).

www.lonza.com/citations

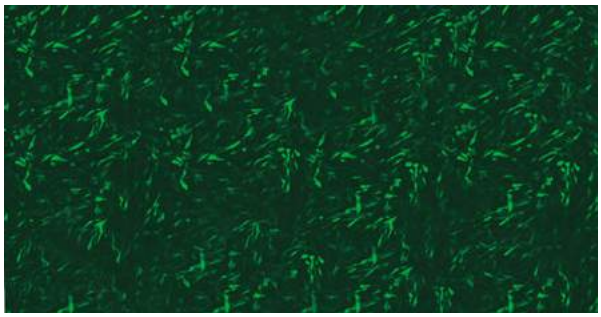
Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-2012	V4XP-2012	P2 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-2024	V4XP-2024	P2 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-2032	V4XP-2032	P2 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-2096	V4SP-2096	P2 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-2960	V4SP-2960	P2 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-2002	V5SP-2002	P2 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-2010	V5SP-2010	P2 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPD-1001	VAPD-1001	Human Dermal Fibroblast Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPD-1001	VPD-1001	Human Dermal Fibroblast Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPD-1001	VVPD-1001	Human Dermal Fibroblast Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
NHDF-Ad – Human Adult Dermal Fibroblasts	61
NHDF-Neo – Neonatal Human Dermal Fibroblasts	61
FGM™ 2 – Fibroblast Growth Media BulletKit™	62

Nucleofector™ Kits for Mouse Embryonic Fibroblasts (MEF)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of MEFs using the different Nucleofection Platforms.
For the transfection of MEFs in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System we recommend using the Primary Cell Optimization Kits and the respective optimization protocols. Optimal Nucleofection Conditions are transferable between these three systems. MEF specific kits are available for the Nucleofector™ II/2b Device.



Example of Nucleofection of primary MEFs. Spontaneously immortalized mouse embryonic fibroblasts (strain: C57BL/6 × 129Sv) were transfected by Nucleofection using a plasmid encoding the enhanced green fluorescent protein eGFP. 24 hours post Nucleofection, the cells were analyzed by fluorescence microscopy. (Photograph courtesy of Dr. H. Hermanns and Prof. P.H. Heinrich, University of Aachen, Germany.)

Benefits

- Transfection efficiency: up to 62%
- Viability: up to 88%

Applications

- Kits suitable for various mouse embryonic fibroblast clones

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VPD-1006	VPD-1006	Mouse Embryonic Fibroblast Starter Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VAPD-1004	VAPD-1004	Mouse Embryonic Fibroblast Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	10 reactions
VAPD-1005	VAPD-1005	Mouse Embryonic Fibroblast Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	10 reactions
VPD-1004	VPD-1004	Mouse Embryonic Fibroblast Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	25 reactions
VPD-1005	VPD-1005	Mouse Embryonic Fibroblast Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	25 reactions
VVPD-1004	VVPD-1004	Mouse Embryonic Fibroblast Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	4 × 25 reactions
VVPD-1005	VVPD-1005	Mouse Embryonic Fibroblast Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Mouse Embryonic Fibroblasts	93
Dulbecco's Modified Eagle's Medium	109

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Nucleofector™ Kits for Mammalian Fibroblasts

For mammalian fibroblasts lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection Conditions.
The P2 and P3 Primary Cell Kits are suited for optimizations of mammalian fibroblasts on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.
A cell group-specific Basic Kit is suited for optimization of mammalian fibroblasts using the Nucleofector™ II/2b Device.

Benefits

- Optimizations can be performed within one experiment
- Detailed protocols guiding through the optimization procedure
- Fine tuning of results is possible with the help of our Scientific Support Team
- Transfection efficiency: up to 90%
- Viability: up to 98%

Applications

- Kits suited for fibroblasts from different mammalian species and various organs
- Already tested for macaque dermal fibroblasts, bovine fibroblasts, human colon myofibroblasts, mouse lung fibroblasts, etc.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-2012	V4XP-2012	P2 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-2024	V4XP-2024	P2 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-2032	V4XP-2032	P2 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-2096	V4SP-2096	P2 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-2960	V4SP-2960	P2 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-2002	V5SP-2002	P2 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-2010	V5SP-2010	P2 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPI-1002	VAPI-1002	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Fibroblasts	100 µL aluminum cuvette	10 reactions
VPI-1002	VPI-1002	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Fibroblasts	100 µL aluminum cuvette	25 reactions
VVPI-1002	VVPI-1002	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Fibroblasts	100 µL aluminum cuvette	4 × 25 reactions

Nucleofector™ Kits for Human Hepatocytes

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human hepatocytes using the different Nucleofection Platforms. Optimal kits for transfection of human hepatocytes in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols.

Benefits

- Transfection efficiency: up to 54%
- Viability: up to 69%
- Cells retain their functionality for up to 120 hours
- Efficient non-viral transfection of non or low proliferating cells

Applications

- Excellent transfection rates for DNA and siRNA
- Study metabolic pathways and toxic effects of new therapeutic agents

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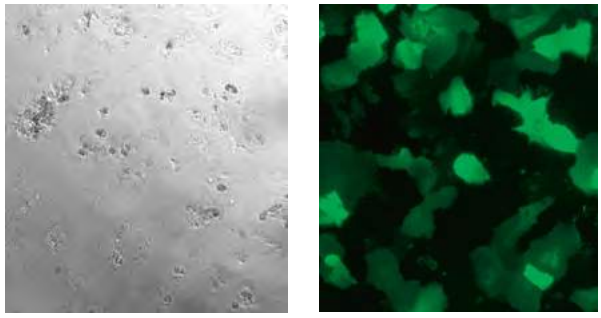

Example showing typical Nucleofection results of human hepatocytes. Cryopreserved human hepatocytes were transfected with pmaxGFP™ Vector. 120 hours post Nucleofection, cells were analyzed by light (A) and fluorescence microscopy (B).

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)

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Nucleofector™ Kits for Mouse or Rat Hepatocytes

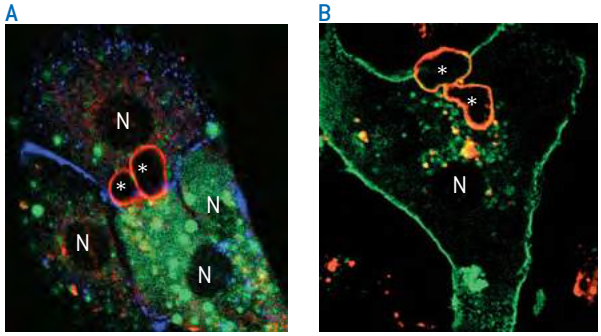
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of mouse or rat hepatocytes using the different Nucleofection Platforms.
For the transfection of mouse or rat hepatocytes in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System we recommend using the Primary Cell Optimization Kits and the respective optimization protocols. Optimal Nucleofection Conditions are transferable between these three systems. Mouse and rat hepatocyte specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 54%
- Viability: up to 80%
- Cells retain functional properties

Applications

- Kits suitable for mouse or rat hepatocytes
- Suited for DNA and siRNA transfections
- Ideal for research on new therapeutic agents and toxicity mechanisms



N = nuclei; * = bile canaliculi

Hepatocytes transfected by Nucleofection maintain their morphology and polarization. Primary rat hepatocytes were transfected by Nucleofection with the pmaxGFP™ Vector (A) or a plasmid containing the cDNA sequence for a plasma membrane receptor-YFP fusion protein (B). Cells were stained with antibodies against desmoplakin (A; blue) to visualize cell boundaries and against multidrug resistance protein 2 (MRP2; A+B; red) to show the apical, canalicular membrane. maxGFP™ Reporter Protein was located in the cytosol of transfected cells (A). YFP-fusion protein was correctly targeted to both the basolateral and the apical membrane domain as shown by co-localization with MRP2 (B). These data prove normal formation of bile canaliculi in hepatocytes transfected by Nucleofection. (Data courtesy of V. Keitel, F. Schliess and D. Häussinger, Department for Gastroenterology, Hepatology and Infectiology, Heinrich-Heine-University Düsseldorf, Germany.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPL-1004	VAPL-1004	Mouse/Rat Hepatocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPL-1004	VPL-1004	Mouse/Rat Hepatocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPL-1004	VVPL-1004	Mouse/Rat Hepatocyte Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Nucleofector™ Kits for Human Aortic Smooth Muscle Cells (AoSMC)

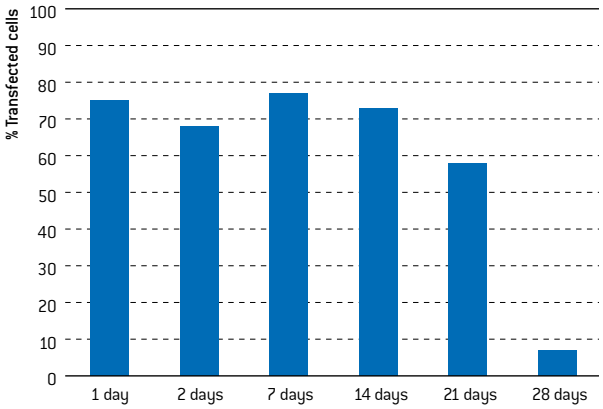
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human AoSMCs using the different Nucleofection Platforms.
Optimal kits for transfection of human AoSMCs in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P1 Primary Cell Kits used in combination with respective basic protocols for mammalian smooth muscle cells. Human AoSMC specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 80%
- Viability: up to 96%
- 10-fold higher transfection efficiency compared to lipofection

Applications

- Kit suitable for human aortic and vascular smooth muscle cells
- Applicable for transient long-term expression up to three weeks
- Ideal tool for studies on human vascular disorders, such as atherosclerosis and stroke



Time course of transient expression of transfected human AoSMC. Clonetics™ Human AoSMC were transfected by Nucleofection using a plasmid encoding the mouse MHC class I heavy chain molecule H-2Kk. 1, 2, 7, 14, 21, and 28 days post Nucleofection, the cells were analyzed for their H-2Kk expression by flow cytometry. Dead cells were excluded from the analysis by propidium iodide staining and gating.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-1012	V4XP-1012	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-1024	V4XP-1024	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-1032	V4XP-1032	P1 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-1096	V4SP-1096	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-1960	V4SP-1960	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-1002	V5SP-1002	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-1010	V5SP-1010	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPC-1001	VAPC-1001	Human Aortic Smooth Muscle Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPC-1001	VPC-1001	Human Aortic Smooth Muscle Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPC-1001	VVPC-1001	Human Aortic Smooth Muscle Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
AoSMC – Human Aortic Smooth Muscle Cells	58
SmGM™ 2 Smooth Muscle Cell Growth Media BulletKit™	56,59
D-AoSMC – Diseased Human Aortic Smooth Muscle Cells (Diabetes Type I or II)	58-64

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Nucleofector™ Kits for Human Skeletal Muscle Myoblasts

Optimal kits for transfection of human skeletal muscle cells (HSMM) in the 4D-Nucleofector™ X Unit are the P5 Primary Cell Kits, used in combination with the cell-type specific protocol. Due to transferability between all platforms, same conditions apply for the 96-well Shuttle™ or 384-well Nucleofector™ Systems.

Benefits

- Transfection efficiency: up to 78%
- Viability: up to 62%

Applications

- Validated to work with Clonetics™ HSMM
- Easily verify previous cell line results in the analogous primary cell type

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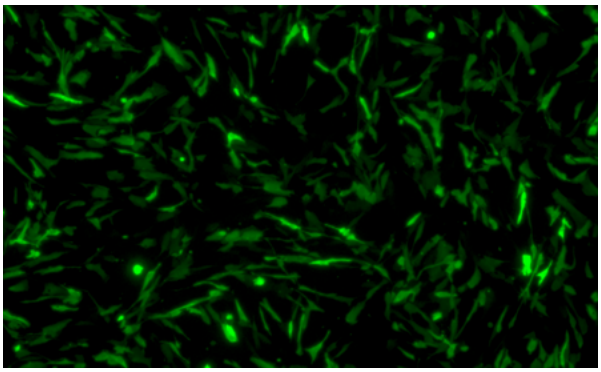

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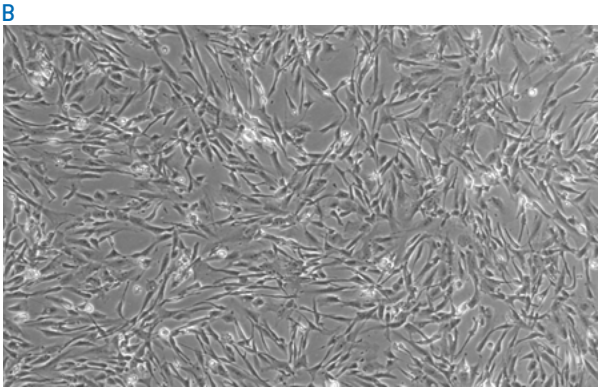


Example of Nucleofection of HSMM. Clonetics™ Human Skeletal Muscle Myoblasts were transfected with pmaxGFP™ Vector. 24 hours post Nucleofection, cells were analyzed by light (A) or fluorescence (B) microscopy.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-5012	V4XP-5012	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-5024	V4XP-5024	P5 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-5032	V4XP-5032	P5 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-5096	V4SP-5096	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-5960	V4SP-5960	P5 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-5002	V5SP-5002	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-5010	V5SP-5010	P5 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)

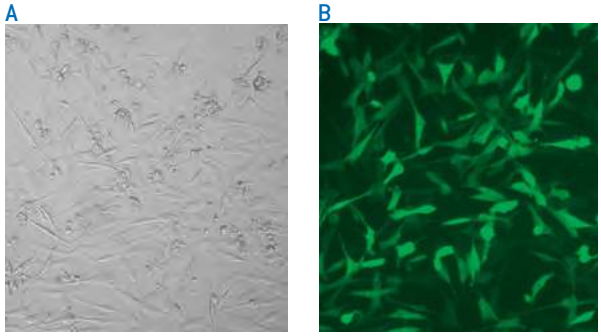
Related Products	Page
HSMM – Human Skeletal Muscle Myoblasts	87
D-HSMM – Diseased Human Skeletal Muscle Myoblasts (Diabetes Type I or II)	87
SkGM™ – Skeletal Muscle Cell Growth Media BulletKit™	87

Nucleofector™ Kits for Mammalian Smooth Muscle Cells

For mammalian smooth muscle cells lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection Conditions.

The P1 Primary Cell Kits together with the cell-group specific Basic Protocols are suited for optimizations of mammalian smooth muscle cells on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.

A cell-group specific Basic Kit is suited for optimization of mammalian smooth muscle cells using the Nucleofector™ II/2b Device.



Nucleofection™ of primary smooth muscle cells. Primary pulmonary artery smooth muscle cells were transfected with pmaxGFP™ Vector. 24 hours post Nucleofection, cells were analyzed by light (A) and fluorescence (B) microscopy.

Benefits

- Optimizations can be performed within one experiment
- Detailed protocols guiding through the optimization procedure
- Fine tuning of results is possible with the help of our Scientific Support Team
- Transfection efficiency: up to 95%
- Viability: up to 96%

Applications

- Kits suited for smooth muscle cells from different mammalian species and various organs
- Already tested for porcine vascular smooth muscle cells and coronary artery smooth muscle cells (Clonetics™ CASMC)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-1012	V4XP-1012	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-1024	V4XP-1024	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-1032	V4XP-1032	P1 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-1096	V4SP-1096	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-1960	V4SP-1960	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-1002	V5SP-1002	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-1010	V5SP-1010	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPI-1004	VAPI-1004	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Smooth Muscle Cells	100 µL aluminum cuvette	10 reactions
VPI-1004	VPI-1004	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Smooth Muscle Cells	100 µL aluminum cuvette	25 reactions
VVPI-1004	VVPI-1004	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Smooth Muscle Cells	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Human Smooth Muscle Cells	58
SmGM™ 2 Smooth Muscle Cell Growth Media BulletKit™	59

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Nucleofector™ Kits for Chicken Neurons

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of chicken neurons using the different Nucleofection Platforms.
Optimal kits for transfection of chicken neurons in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with respective basic protocols for mammalian neurons. Chicken neuron specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 43%
- Transgene expression for more than one week
- Cells retain morphological and functional properties

Applications

- Kits suitable for hippocampal neurons and dorsal root ganglia neurons

New Nucleofection in adherent state is possible using the AD1 4D-Nucleofector™ Y Kit

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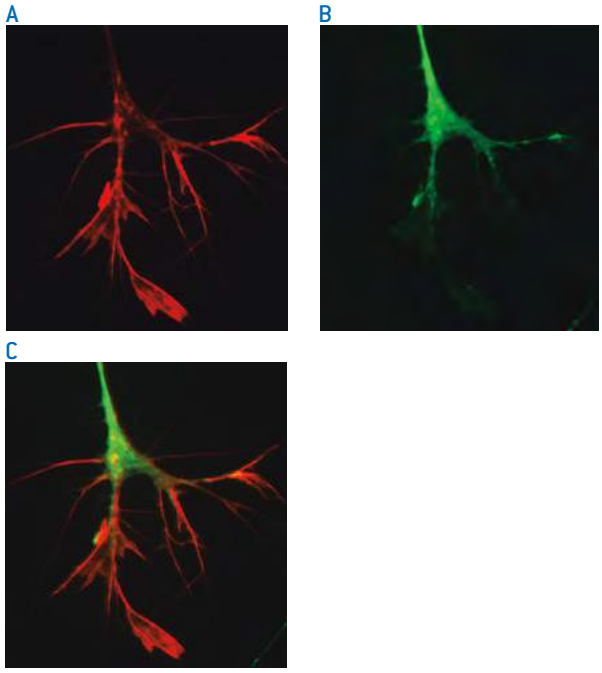


Formation of normal growth cones indicates maintenance of functionality of dorsal root ganglia after Nucleofection. DRG neurons from chicken were transfected by Nucleofection with a plasmid encoding the GFP protein. After cultivation on pre-coated glass coverslips overnight, single cells were analyzed for formation of normal growth cones (A – C), F-actin localization after staining with Alexa 568 conjugated phalloidin (A and C) and GFP expression (B and C). (Photograph courtesy of B. Eickholt, King's College, London, Great Britain.)

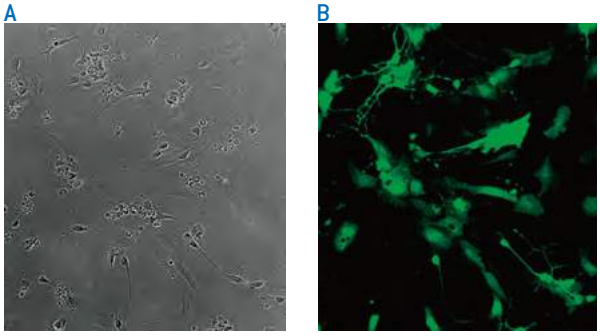
Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VPG-1002	VPG-1002	Chicken Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPG-1002	VVPG-1002	Chicken Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
PNGM™ Primary Neuron Growth Media BulletKit™	96

Nucleofector™ Kits for Mouse Neurons

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of mouse neurons using the different Nucleofection Platforms.
Optimal kits for transfection of mouse neurons in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with respective basic protocols for mammalian neurons. Mouse neuron specific kits are available for the Nucleofector™ II/2b Device.



Nucleofection of primary mouse hippocampal neurons. Primary dissociated neurons of mixed glial cultures were transfected using a plasmid encoding the enhanced green fluorescent protein eGFP. 48 hours post Nucleofection, the cells were analyzed by light (A) and fluorescence microscopy (B). (Photograph courtesy of A. Dityatev, G. Dityateva and M. Hammond, Center for Molecular Neurobiology, Hamburg, Germany.)

Benefits

- Transfection efficiency: up to 60%
- Viability: up to 65 %
- Transgene expression for more than one week
- Cells retain morphological and functional properties

Applications

- Kits suitable for hippocampal, cortical and dorsal root ganglia neurons
- New Nucleofection in adherent state is possible using the AD1 4D-Nucleofector™ Y Kit
- More than 50 peer-reviewed publications

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Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPG-1001	VAPG-1001	Mouse Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPG-1001	VPG-1001	Mouse Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPG-1001	VVPG-1001	Mouse Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Primary Mouse Neural Cells	96
PNGM™ Primary Neuron Growth Media BulletKit™	96

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Nucleofector™ Kits for Rat Neurons

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of rat neurons using the different Nucleofection Platforms.
Optimal kits for transfection of rat neurons in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Rat neuron specific kits are available for the Nucleofector™ II/2b Device.

Benefits

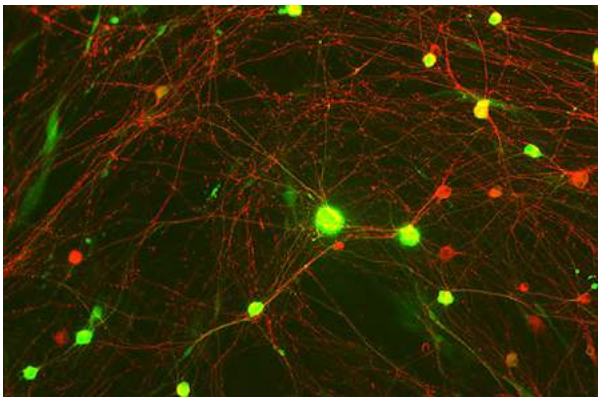
- Transfection efficiency: up to 67% using a non-viral method
- Viability: up to 60%
- Cells retain morphological and functional properties
- Transgene expression for more than one week

Applications

- Kits suitable for hippocampal neurons, cortical neurons and dorsal root ganglia neurons
- Proven performance for siRNA, shRNA, miRNA, and antisense oligonucleotides

New Nucleofection in adherent state is possible using the AD1 4D-Nucleofector™ Y Kit

- Approaching 250 peer-reviewed publications



Cryopreserved dissociated rat DRG cells were thawed and cultured in 24-well plates for Nucleofection using 4D-Nucleofector™ Y Unit. DRG cell culture was transfected at 2 DIV and fixed 24 hours post Nucleofection (program EH-166). Neuronal networks are stained using anti Tuj-1 antibody (red; personal gift W. Staines). Transfected neurons and Schwann cells can be seen in green (maxGFP™ Protein).

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Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPG-1003	VAPG-1003	Rat Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPG-1003	VPG-1003	Rat Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPG-1003	VVPG-1003	Rat Neuron Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Primary Rat Neural Cells	96
PNGM™ Primary Neuron Growth Media BulletKit™	96

Nucleofector™ Kits for Mammalian Neurons

For mammalian neurons lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection Conditions.
The P3 Primary Cell Kits together with the cell-group specific Basic Protocols are suited for optimizations of mammalian neurons on the 4D-Nucleofector™ System, the 96-well Shuttle™ system or the 384-well Nucleofector™ System.
A cell-group specific Basic Kit is suited for optimization of mammalian neurons using the Nucleofector™ II/2b Device.

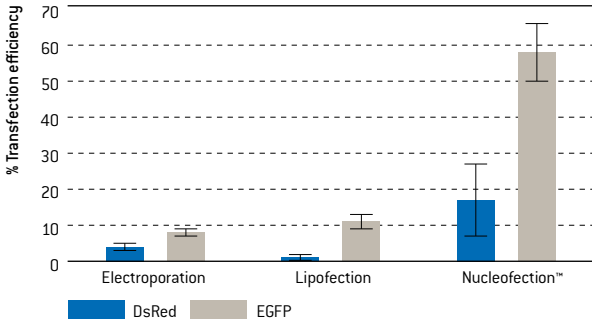
Benefits

- Optimizations can be performed within one experiment
- Detailed protocols provide guidance through the optimization procedure
- Fine tuning of results is possible with the help of our Scientific Support Team
- Transfection efficiency: up to 92%
- Viability: up to 80%

Applications

- Kits suited for various neuron types from different mammalian species

New Nucleofection in adherent state is possible using the AD1 4D-Nucleofector™ Y Kit



Comparison of conventional electroporation, lipofection and Nucleofection for transfection of rat neuronal progenitor cells. Ventral mesencephalic progenitor (VMP) cells from rat brain, which are the important source of dopaminergic neurons for cell replacement strategies in Parkinson's disease, were transfected with two different plasmids expressing DsRed or eGFP. For transfection, conventional electroporation (EasyjecT from EquiBio, 100 µg plasmid per 500,000 cells), lipofection (Lipofectamine™ 2000 Reagent, 0.5 µg DNA per 60,000 cells), or Nucleofection (5 µg DNA per 2,000,000 cells) were used. (Data from Cesnulevicius et al. (2006) Stem Cells 24(12), 2776-91.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPI-1003	VAPI-1003	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Neurons	100 µL aluminum cuvette	10 reactions
VPI-1003	VPI-1003	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Neurons	100 µL aluminum cuvette	25 reactions
VVPI-1003	VVPI-1003	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Neurons	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
PNGM™ Primary Neural Cell Growth Media BulletKit™	96

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Nucleofector™ Kits for Mammalian Glial Cells

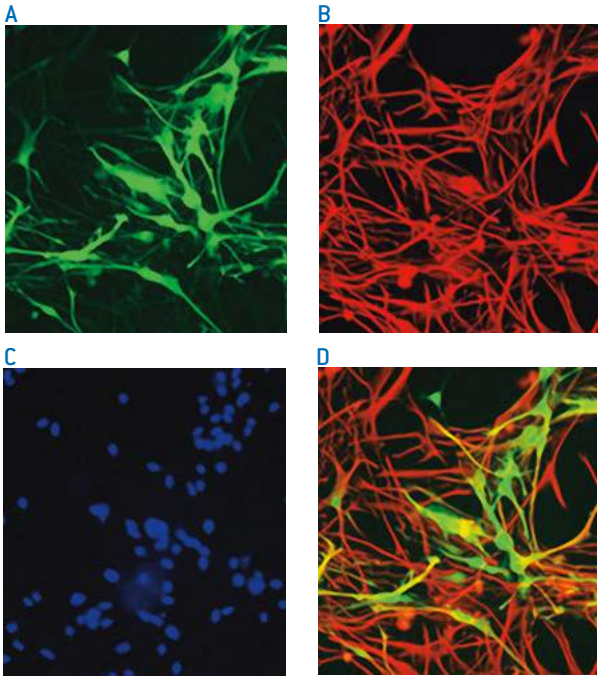
A selection of kits for mammalian glial cells helps you to easily define optimal Nucleofection Conditions.
The P3 Primary Cell Kits together with the cell-group specific Basic Protocols are suited for optimizations of mammalian glial cells on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.
A cell-group specific Basic Kit is suited for optimization of mammalian glial cells using the Nucleofector™ II/2b Device.

Benefits

- Optimizations can be performed within one experiment
- Detailed protocols guide through optimization procedure
- Fine tuning of results is possible with the help of our Scientific Support Team
- Transfection efficiency: up to 67%
- Viability: up to 80%

Applications

- Kits suited for various glial cells from different mammalian species
- Already tested for rat and mouse astrocytes, rat oligodendrocytes
- New Nucleofection in adherent state using the AD1 4D-Nucleofector™ Y Kit



Expression of GFAP and eGFP in rat astrocytes transfected by Nucleofection. Primary rat astrocytes were isolated from rat embryos (E17) and cultured for 10 days until cells reached confluency. These cells were transfected with a plasmid encoding the enhanced green fluorescent protein, eGFP. 24 hours post Nucleofection, cells were analyzed by fluorescence microscopy for expression of eGFP (A) and GFAP (B), an astrocyte-specific marker protein. Nuclei were stained with DAPI (C). (D) shows an overlay of the images. (Photographs courtesy of Dr. Hyun-Ju Kim and Dr. Tim Vartanian, Beth Israel Deaconess Medical Center, Dept. of Neurology, Boston, Massachusetts, USA.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4YP-1A24	V4YP-1A24	AD1 4D-Nucleofector™ Y Kit	24-well Dipping Electrode	24 reactions
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPI-1006	VAPI-1006	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Glial Cells	100 µL aluminum cuvette	10 reactions
VPI-1006	VPI-1006	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Glial Cells	100 µL aluminum cuvette	25 reactions
VVPI-1006	VVPI-1006	Basic Nucleofector™ II/ 2b Kit for Primary Mammalian Glial Cells	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Primary Neural Cells and Media	94-96

Nucleofector™ Kits for Human CD34⁺ Cells

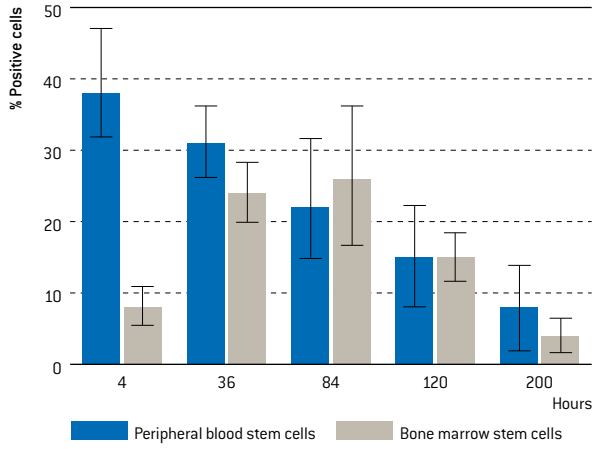
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human CD34⁺ cells using the different Nucleofection Platforms.
Optimal kits for transfection of human CD34⁺ cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Human CD34⁺ cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 83%
- Viability: up to 70%
- No influence on hematopoietic cell differentiation
- Both fresh or cryopreserved material can be used

Applications

- Kits suitable for unstimulated human CD34⁺ bone marrow cells
- Cells can be derived from cord blood or leukapheresis material
- Cited for DNA and siRNA transfection



Long-term transgene expression after Nucleofection of blood and bone marrow derived CD34⁺ cells. Kinetics of deltaLNGFR (Low Affinity Nerve Growth Factor Receptor) expression were determined by flow cytometric analysis. 39 ± 5.9% of peripheral blood stem cells showed deltaLNGFR staining 4 hours after Nucleofection with a continuous decrease (n = 3, 3 patients). Bone marrow stem cells showed maximal deltaLNGFR expression with 26 ± 9.7% 84 hours after transfection, which then decreased in the proliferating culture (n = 3, single patient). (Data courtesy of Greiner et al., University Hospital of Ulm, Ulm, Germany.)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPA-1003	VAPA-1003	Human CD34⁺ Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPA-1003	VPA-1003	Human CD34⁺ Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPA-1003	VVPA-1003	Human CD34⁺ Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Human CD34⁺ Progenitor Cells	22
X-VIVO™ 15 Serum-free Hematopoietic Cell Medium – Chemically Defined	127
HPGM™ Hematopoietic Progenitor Growth Medium	52

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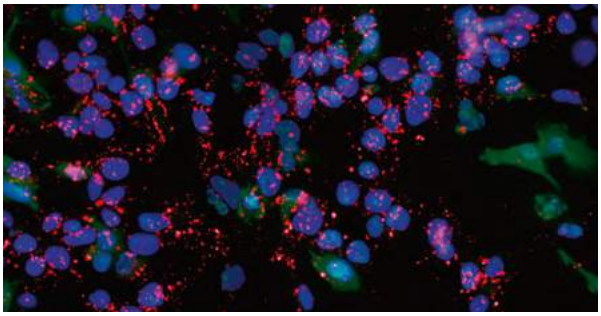
Nucleofector™ Kits for Human H9 Stem Cells

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human H9 stem cells using the different Nucleofection Platforms.

Optimal kits for transfection of human H9 stem cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. A stem cell specific Basic Kit is suited for optimization of human stem cells using the Nucleofector™ II/2b Device.



H9 cells preserve pluripotency post Nucleofection. H9 cells transfected with the pmaxGFP™ Vector maintain their undifferentiated state. Analysis after 24 hours shows expression of maxGFP™ Protein (green) as well as of the pluripotency markers SSEA4 (red) and Oct4 (purple). The blue signals refer to nuclear staining by DAPI. (Data kindly provided by Jennifer Moore, Rutgers University, Piscataway, USA.)

Benefits

- Transfection efficiency: up to 64%
- Viability: up to 98%
- Excellent preservation of pluripotency

Applications

- Kits suitable for human H9 stem cells
- Elucidate various aspects of stem cell differentiation

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VPH-5002	VPH-5002	Human Stem Cell Nucleofector™ II/ 2b Starter Kit	100 µL aluminum cuvette	18 reactions
VAPH-5012	VAPH-5012	Human Stem Cell Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	10 reactions
VPH-5012	VPH-5012	Human Stem Cell Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	25 reactions
VVPH-5012	VVPH-5012	Human Stem Cell Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	4 × 25 reactions
VAPH-5022	VAPH-5022	Human Stem Cell Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	10 reactions
VPH-5022	VPH-5022	Human Stem Cell Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	25 reactions
VVPH-5022	VVPH-5022	Human Stem Cell Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	4 × 25 reactions

Nucleofector™ Kits for Human Mesenchymal Stem Cells (MSC)

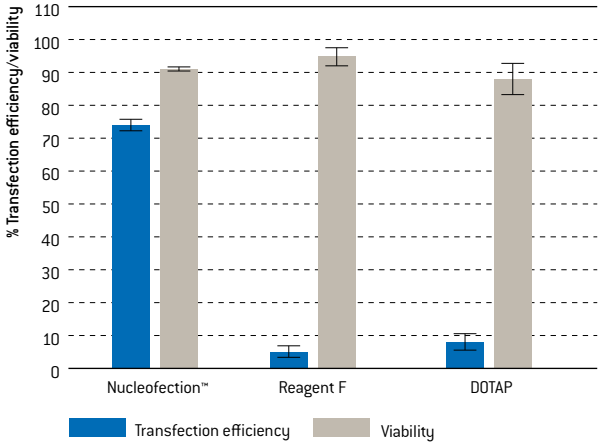
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of human MSC using the different Nucleofection Platforms.
Optimal kits for transfection of human MSC in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P1 Primary Cell Kits used in combination with cell-type specific protocols. Human MSC cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 88%
- Viability: up to 86%
- Maintenance of functional properties
- Efficient non-viral transfection of human MSC

Applications

- Validated to work with Poietics™ MSC
- Differentiation of transfected MSC into adipocytes or osteoblasts



Comparison of Nucleofection with lipofection for transfection of human MSC. MSC were transfected by Nucleofection with pcDNA3/NT-GFP using either Nucleofection or the lipid-based Fugene® 6 or DOTAP Reagents (both Roche Applied Science). MSC transfected by Nucleofection were analyzed for transfection efficiency roughly 60 hours post Nucleofection, cells transfected with Fugene® 6 or DOTAP Reagents were analyzed after 72 hours. Transfection efficiency was scored by flow cytometric analysis and reported as percentage of GFP+ cells. The percentage of viable cells was estimated by trypan blue exclusion. (Data courtesy of Aluigi M, Fogli M, Curti A, Isidori A, Gruppioni E, Chiodoni C, Colombo MP, Versura P, D'Errico-Grigioni A, Ferri E, Baccarani M and Lemoli RM, Institute of Hematology and Medical Oncology, Bologne, Italy).

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-1012	V4XP-1012	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-1024	V4XP-1024	P1 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-1032	V4XP-1032	P1 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-1096	V4SP-1096	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-1960	V4SP-1960	P1 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-1002	V5SP-1002	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-1010	V5SP-1010	P1 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/2b Kits				
VAPE-1001	VAPE-1001	Human Mesenchymal Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPE-1001	VPE-1001	Human Mesenchymal Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPE-1001	VVPE-1001	Human Mesenchymal Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
hMSC Human Mesenchymal Stem Cells	29
MSCGM™ Mesenchymal Stem Cell Growth Medium BulletKit™	29
hMSC Mesenchymal Stem Cell Adipogenic Differentiation BulletKit™	30
hMSC Mesenchymal Stem Cell Osteogenic Differentiation BulletKit™	29
hMSC Mesenchymal Stem Cell Chondrogenic Differentiation BulletKit™	29

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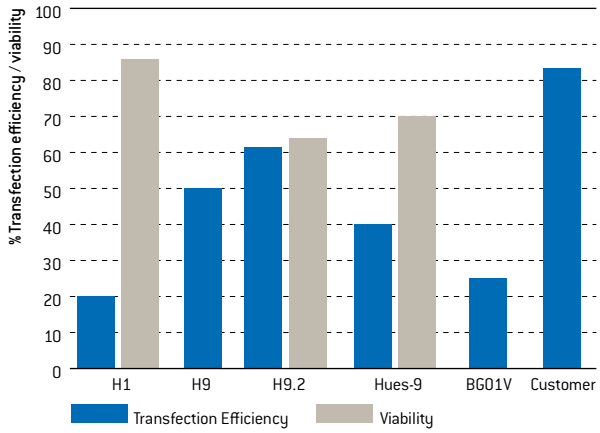
Nucleofector™ Kits for Human Pluripotent Stem Cells

For human pluripotent stem cells (ESC or iPSC), lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection Conditions.
The P3 and P4 Primary Cell Kits together with the cell- group specific Basic Protocols are suited for optimizations of human stem cells on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.
A cell-group specific Basic Kit is suited for optimization of human stem cells using the Nucleofector™ II/2b Device.



Transfection efficiencies of human stem cell lines. Different human stem cell lines were transfected by Nucleofection using the pmaxGFP™ Vector. (Data for Nucleofection of human stem cells are compiled from experiments performed by leading stem cell research customers.)

Benefits

- Transfection efficiency: up to 95%
- Viability: up to 98%
- Circumvents tedious creation of viruses
- Less DNA and lower cell number required

Applications

- Already tested for various human ESC clone (e.g. H1, H9, H14 or HS306) and iPSCs
- Proven for ZFN, TALEN or CRISPR mediated genome editing in human ESCs or iPSCs

www.lonza.com/genome-editing

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4XP-4012	V4XP-4012	P4 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-4024	V4XP-4024	P4 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-4032	V4XP-4032	P4 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
V4SP-4096	V4SP-4096	P4 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-4960	V4SP-4960	P4 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
V5SP-4002	V5SP-4002	P4 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-4010	V5SP-4010	P4 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VPH-5002	VPH-5002	Human Stem Cell Nucleofector™ II/ 2b Starter Kit	100 µL aluminum cuvette	18 reactions
VAPH-5012	VAPH-5012	Human Stem Cell Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	10 reactions
VPH-5012	VPH-5012	Human Stem Cell Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	25 reactions
VVPH-5012	VVPH-5012	Human Stem Cell Nucleofector™ II/ 2b Kit 1	100 µL aluminum cuvette	4 × 25 reactions
VAPH-5022	VAPH-5022	Human Stem Cell Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	10 reactions
VPH-5022	VPH-5022	Human Stem Cell Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	25 reactions
VVPH-5022	VVPH-5022	Human Stem Cell Nucleofector™ II/ 2b Kit 2	100 µL aluminum cuvette	4 × 25 reactions

Nucleofector™ Kits for iPSC Generation

The Nucleofector™ Technology has been demonstrated to be an efficient and cost-effective non-viral alternative for iPSC generation and is being used by leading scientists around the world.

Benefits

- Simple, single-step procedure to introduce DNA/RNA, e.g. episomal vectors
- Successfully tested for generation of iPSCs from various cell types
- Seamless combination with our L7™ hPSC Culture System for feeder- and xeno-free iPSC culture
- Availability of 4D-Nucleofector™ Kits with GMP Solutions

Applications

- Reprogramming of various cell types, including PBMCs, CD34⁺ hematopoietic stem cells or fibroblasts

To determine the required Nucleofector™ Kit for your reprogramming cell type of interest please refer to the respective Nucleofector™ Kit page.

Selected Publications	
Fibroblast	Arnold *et al.* (2012) ISRN Cell Biol, Article ID 124878
	Chen *et al.* (2013) PLoS ONE 8(10): e75682
	Goyal *et al.* (2013) PLoS ONE 8(12): e82838
	Mehta A *et al.* (2011) Cardiovasc Res 91:577-86
	Olsen *et al.* (2012) Lonza Resource Notes, Fall: 9-12
	Yu J *et al.* (2009) Science 324(5928):797-801
CD34⁺ Cells	Ben Nun IF (2013) Lonza Resource Notes, Spring: 8-11
	Chou BK *et al.* (2011) Cell Research 21:518-529
	Mack A *et al.* (2011) PloS ONE 6(11): e27956
	Margariti *et al* (2012) PNAS 109(34):13793–13798
	Yu J *et al.* (2011) PLoS ONE 6(3): e17557
BMMCs or PBMCs	Chou BK *et al.* (2011) Cell Research 21:518-529
	Hu K *et al.* (2011) Blood 117(14): e109-e119
ADSCs	Jia *et al.* (2010) Nature Methods 7:197-199
	Yu J *et al.* (2011) PLoS ONE 6(3): e17557
DPSCs	Chen *et al.* (2013) PLoS ONE 8(10): e75682
MEFs	Kaji K *et al.* (2009) Nature 458(7239):771-775

L7™ PBMC Reprogramming Bundle

Using the 4D-Nucleofector™ System, Lonza's Pluripotent Stem Cell Innovation Team has developed an optimized protocol for reprogramming of peripheral blood mononuclear cells (PBMCs) using episomal vectors.

Benefits

- Ready-to-use reprogramming protocol for reliable reprogramming of PBMCs
- Seamless combination with our L7™ hPSC Culture System for feeder- and xeno-free iPSC culture

Applications

- Reprogramming of PBMCs using the 4D-Nucleofector™ System

Ordering Information

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
CC-2702	CC-2702	hPBMC – Human Peripheral Blood Mononuclear Cells	Cryopreserved, volume discount available	≥50 million cells/vial
FP-5124	FP-5124	L7™ PBMC Priming-Recovery BulletKit	Containing: L7™ PBMC Priming-Recovery Basal Medium, L7™ PBMC Reprogramming Enhancers A and B	10 reactions
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions

Related Products	Page
Human Peripheral Blood Mononuclear Cells	21
4D-Nucleofector™ System	175
L7™ hPSC Culture System	35

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Nucleofector™ Kits for Mouse Embryonic Stem (ES) Cells

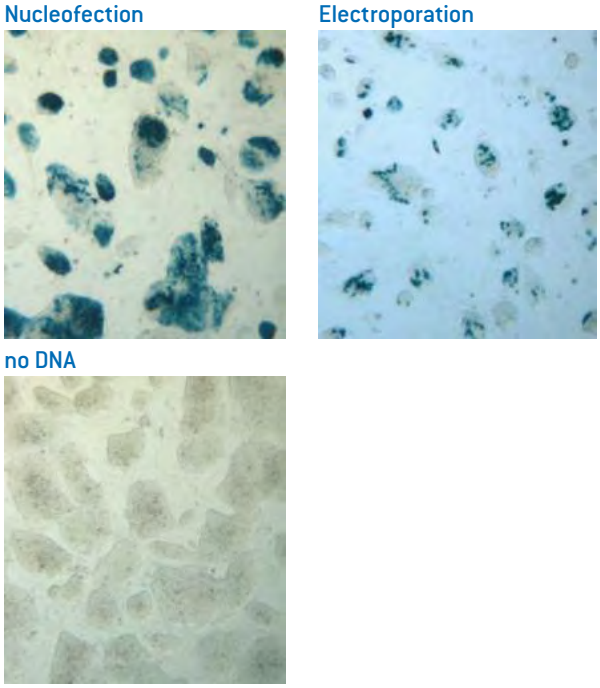
Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of mouse ES cells using the different Nucleofection Platforms.
Optimal kits for transfection of mouse ES cells in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System are the P3 Primary Cell Kits used in combination with cell-type specific protocols. Mouse ES cell specific kits are available for the Nucleofector™ II/2b Device.

Benefits

- Transfection efficiency: up to 90%
- Viability: up to 99%
- Homogenous transient gene expression pattern
- Preservation of cell functionality (ability to differentiate)

Applications

- Tested with several mouse ES cell lines (e.g., R1, D3, E14)
- Successfully used to generate germline chimeras



Comparison of Nucleofection and electroporation for transfection of mouse ES cells. Mouse ES cells were transfected by Nucleofection and compared to mock-transfected (no DNA) and electroporated ES cells using Bio-Rad® Gene Pulser®. Cells were stained 48 hours after transfection for transient lacZ expression. (Data courtesy of S. Boljahn, A. Rode, M. Joao da Silva, T. Hennek and B. Zevnik, Artemis Pharmaceutical GmbH, Cologne, Germany.)

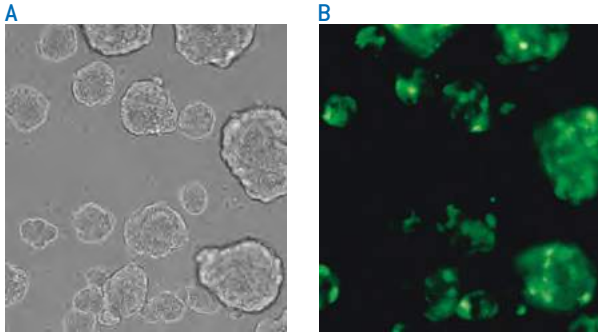
Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-3012	V4XP-3012	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XP-3024	V4XP-3024	P3 Primary Cell 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XP-3032	V4XP-3032	P3 Primary Cell 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-3096	V4SP-3096	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SP-3960	V4SP-3960	P3 Primary Cell 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-3002	V5SP-3002	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SP-3010	V5SP-3010	P3 Primary Cell 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPH-1001	VAPH-1001	Mouse Embryonic Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPH-1001	VPH-1001	Mouse Embryonic Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPH-1001	VVPH-1001	Mouse Embryonic Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Related Products	Page
Mouse Embryonic Fibroblasts (as feeder cells)	93
DMEM 4.5 g/L glucose with L-Glutamine	109

Nucleofector™ Kits for Mouse Neural Stem Cells (NSC)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of mouse NSC using the different Nucleofection Platforms.
For the transfection of mouse NSC in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System we recommend using the Primary Cell Optimization Kits and the respective optimization protocols. Optimal Nucleofection Conditions are transferable between these three systems. Mouse NSC specific kits are available for the Nucleofector™ II/2b Device.



Nucleofection of mouse NSCs. Primary NSCs isolated from the lateral ventrical wall of an adult mouse were transfected by Nucleofection using a plasmid encoding the enhanced green fluorescent protein eGFP. 48 hours post Nucleofection, the cells were analyzed by light (A) and fluorescence microscopy (B). (Photograph courtesy of Dr. L. Wikstrom et al., NeuroNova, Stockholm, Sweden.)

Benefits

- Transfection efficiency: up to 82%
- Viability: up to 90%
- Transgene expression for several days

Applications

- Kits suitable for mouse neurospheres and adherent cells
- Differentiation into neurons and astrocytes possible

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VAPG-1004	VAPG-1004	Mouse Neural Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	10 reactions
VPG-1004	VPG-1004	Mouse Neural Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPG-1004	VVPG-1004	Mouse Neural Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

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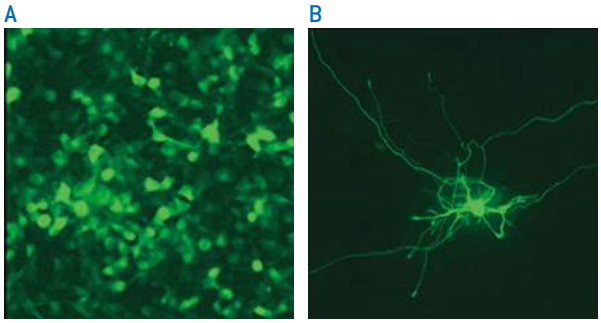
Nucleofector™ Kits for Rat Neural Stem Cells (NSC)

Various Nucleofector™ Kits and corresponding Optimized Protocols are available for the transfection of rat NSC using the different Nucleofection Platforms.
For the transfection of rat NSC in the 4D-Nucleofector™, 96-well Shuttle™ or 384-well Nucleofector™ System we recommend using the Primary Cell Optimization Kits and the respective optimization protocols. Optimal Nucleofection Conditions are transferable between these three systems. Rat NSC specific kits are available for the Nucleofector™ II/2b Device.



Nucleofection of rat NSC. Primary NSC isolated from rat embryos (E14) were transfected by Nucleofection using a plasmid encoding enhanced green fluorescent protein eGFP under control of an EF1alpha promoter (pcDNAEF1-eGFP). Post Nucleofection, cells were cultured with bFGF for 2 days, then for 5 additional days without bFGF to differentiate into neurons. Cells were analyzed 2 days (A) and 7 days (B) post Nucleofection by fluorescence microscopy. (Photograph courtesy of S.H. Lee, College of Medicine, Dept. of Biochemistry, Hanyang University, Seoul, South Korea.)

Benefits

- Transfection efficiency: up to 46%
- Efficient non-viral method for efficient gene transfer into primary neural stem cells
- Transgene expression for several days

Applications

- Kits suitable for rat neurospheres and adherent cells
- Differentiation into neurons and astrocytes possible

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)
Nucleofector™ II/ 2b Kits				
VPG-1005	VPG-1005	Rat Neural Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	25 reactions
VVPG-1005	VVPG-1005	Rat Neural Stem Cell Nucleofector™ II/ 2b Kit	100 µL aluminum cuvette	4 × 25 reactions

Nucleofector™ Kits for Animal Stem Cells

For animal stem cells cells lacking a cell-type specific Optimized Protocol, we offer a selection of kits that can be used to easily define optimal Nucleofection Conditions.
The Primary Cell Optimization Kits are suited for optimizations of stem cells on the 4D-Nucleofector™ System, the 96-well Shuttle™ System or the 384-well Nucleofector™ System.

Benefits

- Protocols provide guidance through the optimization procedure
- Optimizations can be performed within one experiment
- Fine tuning of results is possible with the help of our Scientific Support Team

Applications

- Kits suited for stem cells from different mammalian species and various organs

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
4D-Nucleofector™ Kits				
V4XP-9096	V4XP-9096	Primary Cell Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	96 reactions (16-well)
96-well Shuttle™ Kits				
V4SP-9096	V4SP-9096	Primary Cell Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	160 reactions (96-well)
384-well Nucleofector™ Kits				
V5SP-9001	V5SP-9001	Primary Cell Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)

Related Products	Page
Nucleofector™ Kits for Human Stem Cells	225,227

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Cell Line Kits for 4D-Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

We offer three different Cell Line Nucleofector™ Solutions SE, SF and SG for the 4D-Nucleofector™,X Unit, 96-well Shuttle™ and the 384-well Nucleofector™ Systems.

Each Cell Line Kit Contains

- Specific Nucleofector™ Solution SE, SF or SG
- Supplement
- pmaxGFP™ Control Vector
- Either single 100 µL Nucleocuvettes™, 16-well Nucleocuvette™ Strips, 96-well or 384-well Nucleocuvette™ Plates

All kits are available in various sizes (please refer to ordering information for details). Optimized Protocols outlining the optimal Nucleofector™ Kit for a broad selection of cell lines are available and can be downloaded from our website. You can always find the most up-to-date information in our online cell database.

Benefits

- Each of the three Nucleofector™ Solutions can be used for a selection of different cell lines
- Conditions are transferable between 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems and between 20 and 100 µL Nucleocuvettes™

Applications

- Transfection of lower cell numbers (from 2×10^4 to 1×10^6 cells) to higher cell numbers (from 2×10^5 to 2×10^7 cells) is possible
- Flexible throughput from single cuvette (100 µL) to 16-well Nucleocuvette™ Strip (20 µL), 96-well and 384-well Nucleocuvette™ Plates is possible

www.lonza.com/celldatabase
www.lonza.com/protocols

100 µL Nucleocuvette™ (4D-Nucleofector™ System)	16-well Nucleocuvette™ Strip (4D-Nucleofector™ System)	96-well Nucleocuvette™ Plate (96-well Shuttle™ System)	384-well Nucleocuvette™ Plate (384-well Nucleofector™ System)

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Cell Line Kits for 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems				
V4XC-1012	V4XC-1012	SE Cell Line 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XC-1024	V4XC-1024	SE Cell Line 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XC-1032	V4XC-1032	SE Cell Line 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4XC-2012	V4XC-2012	SF Cell Line 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	12 reactions
V4XC-2024	V4XC-2024	SF Cell Line 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XC-2032	V4XC-2032	SF Cell Line 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
V4XC-3012	V4XC-3012	SG Cell Line 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™	12 reactions
V4XC-3024	V4XC-3024	SG Cell Line 4D-Nucleofector™ X Kit L	100 µL Nucleocuvette™ Vessel	24 reactions
V4XC-3032	V4XC-3032	SG Cell Line 4D-Nucleofector™ X Kit S	20 µL Nucleocuvette™ Strip	32 reactions (16-well)
Cell Line Kits for 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems				
V4SC-1096	V4SC-1096	SE Cell Line 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SC-1960	V4SC-1960	SE Cell Line 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
V4SC-2096	V4SC-2096	SF Cell Line 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SC-2960	V4SC-2960	SF Cell Line 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)
V4SC-3096	V4SC-3096	SG Cell Line 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
V4SC-3960	V4SC-3960	SG Cell Line 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	960 reactions (96-well)

Cell Line Kits for 4D-Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

Continued

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Cell Line Kits for 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems				
V5SC-1002	V5SC-1002	SE Cell Line 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SC-1010	V5SC-1010	SE Cell Line 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
V5SC-2002	V5SC-2002	SF Cell Line 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SC-2010	V5SC-2010	SF Cell Line 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)
V5SC-3002	V5SC-3002	SG Cell Line 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	768 reactions (384-well)
V5SC-3010	V5SC-3010	SG Cell Line 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	3840 reactions (384-well)

Quick Reference Guide – Cell Line Kits

				Kits for 4D-Nucleofector™ (Cat. No.)			Kits for 96-well Shuttle™ (Cat. No.)	
Cell line	Efficiency	Viable cells	Solution	100 µL (12 rxn) Cat. No.	100 µL (24 rxn) Cat. No.	20 µL (32 rxn) Cat. No.	20 µL (96 rxn) Cat. No.	20 µL (960 rxn) Cat. No.
293	83%	93%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
3T3-L1 pre-ad	97%	66–79%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
A20	80%		SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
A549	81%	62%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
ARPE-19	87%		SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
Ba/F3	80%	60–70%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
Beta TC-6	66–77%	49–82%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
BHK-21	97–98%	91–95%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
C6	92%	55–70%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
CHO-K1	86%	97%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
CHO-S [suspension]	86%	55–57%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
COS-7	91–99%	80–96%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
DU 145	89%	86–92%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
EL4	70–80%		SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
GH3	60–80%	60–70%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
H9C2	80–90%	54–72%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
HCT 116	70–80%	65–75%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
HeLa	75%	89%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
HeLa S3	61–85%	62–95%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
Hep G2	95.50%	92.70%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
HL-60	58%	61%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
HT29	51–67%	60%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
IMR32	74–86%	45–63%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
IMR90	65%	70%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
Jurkat	92%	71–80%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
K-562	92%	95%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
L-428	70–80%	85%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
LnCAP	70%	45%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
MCF7	72%	89%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
MDA-MB-231	73–89%		SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
MDCK	72–82%	50–55%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
MG63	70–73%	60–65%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
MRC-5	84–86%	67–73%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960

Cell Line Kits for 4D Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

Continued

Quick Reference Guide – Cell Line Kits

Cell line	Efficiency	Viable cells	Solution	Kits for 4D-Nucleofector™ (Cat. No.)			Kits for 96-well Shuttle™ (Cat. No.)	
				100 µL (12 rxn) Cat. No.	100 µL (24 rxn) Cat. No.	20 µL (32 rxn) Cat. No.	20 µL (96 rxn) Cat. No.	20 µL (960 rxn) Cat. No.
Neuro-2a [N2a]	67%	82%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
NIH / 3T3	95%	93%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
PC3	83%	79%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
Raji	65–69%	71%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
Ramos	40–51%	70–77%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
RAW 264.7	60%	86%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
RIN-m5F	68–90%	71–85%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
Sf9	100%	48–64%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
SH-SY5Y	81%	80%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
Sp2-0	65–69%	80–90%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
T-47D	80%		SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
T84	88%	50–70%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960
THP-1	65%	81%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
U-87MG	75%	40–50%	SE	V4XC-1012	V4XC-1024	V4XC-1032	V4SC-1096	V4SC-1960
U-937	36%	85%	SG	V4XC-3012	V4XC-3024	V4XC-3032	V4SC-3096	V4SC-3960
Vero	92%	80–95%	SF	V4XC-2012	V4XC-2024	V4XC-2032	V4SC-2096	V4SC-2960

Cell Line Optimization Kits for 4D Nucleofector™, X Unit, 96-well Shuttle™ and 384-well Nucleofector™ Systems

The Cell Line Optimization Nucleofector™ Kits are the ideal tool to conveniently and rapidly determine the optimal Nucleofection Condition of virtually any difficult-to-transfect cell line within one experiment.
With the unique capability of the different Nucleofector™ Platforms (4D-Nucleofector™ System, 96-well Shuttle™ System or 384-well Nucleofector™ System) to address individual wells of a 16-well, 96-well or 384-well Nucleocuvette™ Plate with different programs, cell line optimizations are easily performed within one experiment. In each system our three Cell Line Nucleofector™ Solutions SE, SF and SG are tested with a pre-selected set of programs plus controls.

Benefits

– Optimal Nucleofection Conditions determined on one platform are transferable to the others and also to the 100 µL single Nucleocuvette™ on the 4D-Nucleofector X Unit

Application

– Convenient and rapid determination of optimal Nucleofection Conditions for virtually any difficult-to-transfect cell line within 1 experiment

Platform	4D-Nucleofector™ System	96-well Shuttle™ System	384-well Nucleofector™ System
Kit contents	- Four 16-well Nucleocuvette™ Strips - Specific Nucleofector™ Solution - Supplement - pmaxGFP™ Control Vector	- One 96-well Nucleocuvette™ Plate - Specific Nucleofector™ Solution - Supplement - pmaxGFP™ Control Vector	- One 384-well Nucleocuvette™ Plate - Specific Nucleofector™ Solution - Supplement - pmaxGFP™ Control Vector
Number of optimization reactions	48 rxn (plus 16 rxn for optional fine tuning)	96 rxn	384 rxn

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Cell Line Kits for 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems				
V4XC-9064	V4XC-9064	Cell Line Optimization 4D-Nucleofector™ X Kit	20 µL Nucleocuvette™ Strip	64 reactions (16-well)
Cell Line Kits for 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems				
V4SC-9096	V4SC-9096	Cell Line Optimization 96-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	96 reactions (96-well)
Cell Line Kits for 4D-Nucleofector™, 96-well Shuttle™ and 384-well Nucleofector™ Systems				
V5SC-9001	V5SC-9001	Cell Line Optimization 384-well Nucleofector™ Kit	20 µL Nucleocuvette™ Plate	384 reactions (384-well)

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

Cell Line Kits for Nucleofector™ II/2b Device

For the transfection of cell lines with the Nucleofector™ II/2b Device, we offer five different Cell Line Nucleofector™ Solutions: C, L, R, T, and V. Optimized Protocols outlining the optimal Nucleofector™ Kit for a large selection of cell lines are available and can be downloaded from our website.

Benefits

– Achieve transfection efficiencies of up to 90% with high cell viability

Applications

– Get up to 99% transfection efficiency with siRNA duplexes even in suspension cells
– Expression within hours – from transfection to analysis in a day

www.lonza.com/celldatabase

www.lonza.com/protocols

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Size
VACA-1004	VACA-1004	Cell Line Nucleofector™ Kit C	10 reactions
VCA-1004	VCA-1004	Cell Line Nucleofector™ Kit C	25 reactions
VVCA-1004	VVCA-1004	Cell Line Nucleofector™ Kit C	4 × 25 reactions
VACA-1005	VACA-1005	Cell Line Nucleofector™ Kit L	10 reactions
VCA-1005	VCA-1005	Cell Line Nucleofector™ Kit L	25 reactions
VVCA-1005	VVCA-1005	Cell Line Nucleofector™ Kit L	4 × 25 reactions
VACA-1001	VACA-1001	Cell Line Nucleofector™ Kit R	10 reactions
VCA-1001	VCA-1001	Cell Line Nucleofector™ Kit R	25 reactions
VVCA-1001	VVCA-1001	Cell Line Nucleofector™ Kit R	4 × 25 reactions
VACA-1002	VACA-1002	Cell Line Nucleofector™ Kit T	10 reactions
VCA-1002	VCA-1002	Cell Line Nucleofector™ Kit T	25 reactions
VVCA-1002	VVCA-1002	Cell Line Nucleofector™ Kit T	4 × 25 reactions
VACA-1003	VACA-1003	Cell Line Nucleofector™ Kit V	10 reactions
VCA-1003	VCA-1003	Cell Line Nucleofector™ Kit V	25 reactions
VVCA-1003	VVCA-1003	Cell Line Nucleofector™ Kit V	4 × 25 reactions

Quick Reference Guide – Optimized Protocols for Nucleofector™ II/2b Device – Cell Lines

Cell line	Efficiency	Viable cells	Solution	10 rxn	25 rxn	100 rxn
293	84%		V	VACA-1003	VCA-1003	VVCA-1003
32D	79%	61%	V	VACA-1003	VCA-1003	VVCA-1003
3T3-L1 ad	25%	90%	L	VACA-1005	VCA-1005	VVCA-1005
3T3-L1 pre-ad	73%	59%	V	VACA-1003	VCA-1003	VVCA-1003
A-10	64%	74%	L	VACA-1005	VCA-1005	VVCA-1005
A-375	72%	97%	V	VACA-1003	VCA-1003	VVCA-1003
A-431	45%	83%	T	VACA-1002	VCA-1002	VVCA-1002
A20	37–74%	81–95%	V	VACA-1003	VCA-1003	VVCA-1003
A2058	81%	94%	C	VACA-1004	VCA-1004	VVCA-1004
A549	72%	81%	T	VACA-1002	VCA-1002	VVCA-1002
A7r5	49%	81%	V	VACA-1003	VCA-1003	VVCA-1003
AGS	73%	62%	V	VACA-1003	VCA-1003	VVCA-1003
ARPE-19	83%	92%	V	VACA-1003	VCA-1003	VVCA-1003

Quick Reference Guide – Optimized Protocols for Nucleofector™ II/2b Device – Cell Lines

Cell line	Efficiency	Viable cells	Solution	10 rxn	25 rxn	100 rxn
B16-F0	84%	91%	R	VACA-1001	VCA-1001	VVCA-1001
B16-F10	91%	96%	V	VACA-1003	VCA-1003	VVCA-1003
BA/F3	88%	79%	V	VACA-1003	VCA-1003	VVCA-1003
BHK-21	85%	78%	L	VACA-1005	VCA-1005	VVCA-1005
BJ	52%	76%	R	VACA-1001	VCA-1001	VVCA-1001
BxPC-3	28%	62%	L	VACA-1005	VCA-1005	VVCA-1005
C2C12	82%	93%	V	VACA-1003	VCA-1003	VVCA-1003
C6	94%	75–80%	V	VACA-1003	VCA-1003	VVCA-1003
Caco-2	59%	70%	T	VACA-1002	VCA-1002	VVCA-1002
Capan-1	29%	78%	V	VACA-1003	VCA-1003	VVCA-1003
CCRF-CEM	68%	79%	C	VACA-1004	VCA-1004	VVCA-1004
CHO (suspension)	92%	82%	V	VACA-1003	VCA-1003	VVCA-1003
CHO-K1	94%	53–87%	T	VACA-1002	VCA-1002	VVCA-1002
CHO-S (suspension)	90–98%	67–72%	V	VACA-1003	VCA-1003	VVCA-1003
COS-1	49%	64%	T	VACA-1002	VCA-1002	VVCA-1002
COS-7	99%	94%	R	VACA-1001	VCA-1001	VVCA-1001
D1 ORL UVA	61%	97%	T	VACA-1002	VCA-1002	VVCA-1002
DU 145	47%	89%	L	VACA-1005	VCA-1005	VVCA-1005
EL4	65%	76%	L	VACA-1005	VCA-1005	VVCA-1005
FDC-P1	82%	84%	L	VACA-1005	VCA-1005	VVCA-1005
GH3	77%	84%	L	VACA-1005	VCA-1005	VVCA-1005
H9c2(2-1)	86%	90%	L	VACA-1005	VCA-1005	VVCA-1005
HaCaT	43%		V	VACA-1003	VCA-1003	VVCA-1003
HCT 116	78%	76%	V	VACA-1003	VCA-1003	VVCA-1003
HeLa	70%		R	VACA-1001	VCA-1001	VVCA-1001
HeLa S3	67%	95%	L	VACA-1005	VCA-1005	VVCA-1005
Hep G2	41–64%	86–94%	V	VACA-1003	VCA-1003	VVCA-1003
HL-60	90%	50–65%	V	VACA-1003	VCA-1003	VVCA-1003
HT-1080	74%	76%	T	VACA-1002	VCA-1002	VVCA-1002
HT-29	16–51%	57–94%	R	VACA-1001	VCA-1001	VVCA-1001
HuT 78	53%	64%	R	VACA-1001	VCA-1001	VVCA-1001
HUV-EC-C	75%	77%	V	VACA-1003	VCA-1003	VVCA-1003
IMR-32	80%	62%	L	VACA-1005	VCA-1005	VVCA-1005
IMR-90	51%	70%	R	VACA-1001	VCA-1001	VVCA-1001
Jurkat	65–80%	74%	V	VACA-1003	VCA-1003	VVCA-1003
K-562	79%	89%	V	VACA-1003	VCA-1003	VVCA-1003
KG-1	70%	84%	R	VACA-1001	VCA-1001	VVCA-1001
KG-1a	86%	79%	L	VACA-1005	VCA-1005	VVCA-1005
L-428	78%	73%	L	VACA-1005	VCA-1005	VVCA-1005
L6	59%	92%	R	VACA-1001	VCA-1001	VVCA-1001
LNCaP	82%	70–80%	R	VACA-1001	VCA-1001	VVCA-1001
MCF7	77%	60%	V	VACA-1003	VCA-1003	VVCA-1003
MDA-MB-231	79%	77%	V	VACA-1003	VCA-1003	VVCA-1003
MDA-MB-453	54%	90%	C	VACA-1004	VCA-1004	VVCA-1004
MDA-MB-468	60%	81%	V	VACA-1003	VCA-1003	VVCA-1003
MDBK	59%	96%	R	VACA-1001	VCA-1001	VVCA-1001
MDCK	73%	83%	L	VACA-1005	VCA-1005	VVCA-1005
MDCK II	80%	88%	L	VACA-1005	VCA-1005	VVCA-1005

Quick Reference Guide – Optimized Protocols for Nucleofector™ II/2b Device – Cell Lines

Cell line	Efficiency	Viable cells	Solution	10 rxn	25 rxn	100 rxn
MEG-01	80%	66%	C	VACA-1004	VCA-1004	VVCA-1004
MG-63	62%	90%	C	VACA-1004	VCA-1004	VVCA-1004
MOLT-4	55%	61%	L	VACA-1005	VCA-1005	VVCA-1005
MV-4-11	29%	79%	L	VACA-1005	VCA-1005	VVCA-1005
NALM-6	64%	87%	T	VACA-1002	VCA-1002	VVCA-1002
NB-4	71%	66%	V	VACA-1003	VCA-1003	VVCA-1003
NCI-H1299 (H1299)	99%	75%	C	VACA-1004	VCA-1004	VVCA-1004
NCTC clone 929	67%	91%	V	VACA-1003	VCA-1003	VVCA-1003
Neuro-2a (N2a)			V	VACA-1003	VCA-1003	VVCA-1003
NG108-15	64%	82%	V	VACA-1003	VCA-1003	VVCA-1003
NIH/$_3$T3	84%	87–89%	R	VACA-1001	VCA-1001	VVCA-1001
NK-92	26%	40%	R	VACA-1001	VCA-1001	VVCA-1001
NRK	44%	91%	T	VACA-1002	VCA-1002	VVCA-1002
NS0	83%	54%	C	VACA-1004	VCA-1004	VVCA-1004
NTERA-2 cl.D1	90%	94%	L	VACA-1005	VCA-1005	VVCA-1005
P19	85%	80%	V	VACA-1003	VCA-1003	VVCA-1003
P815	62%	92%	T	VACA-1002	VCA-1002	VVCA-1002
PANC-1	68%	75%	R	VACA-1001	VCA-1001	VVCA-1001
PC-12	92%	81%	V	VACA-1003	VCA-1003	VVCA-1003
PC-3	88%	59–66%	V	VACA-1003	VCA-1003	VVCA-1003
Raji	84%	67–81%	V	VACA-1003	VCA-1003	VVCA-1003
Ramos	27%	72%	V	VACA-1003	VCA-1003	VVCA-1003
RAW 264.7	65%	74%	V	VACA-1003	VCA-1003	VVCA-1003
RBL-1	83%	67%	V	VACA-1003	VCA-1003	VVCA-1003
RBL-2H3	42%	93%	T	VACA-1002	VCA-1002	VVCA-1002
S49	81%	68–95%	V	VACA-1003	VCA-1003	VVCA-1003
Saos-2	82%	79%	V	VACA-1003	VCA-1003	VVCA-1003
Schneider's Drosophila Line 2	77%	64–70%	V	VACA-1003	VCA-1003	VVCA-1003
Sf9	82%	76–79%	R	VACA-1001	VCA-1001	VVCA-1001
SH-SY5Y	63–82%	40%	V	VACA-1003	VCA-1003	VVCA-1003
SK-BR-3	50%	94%	C	VACA-1004	VCA-1004	VVCA-1004
SK-N-SH	85%	73%	V	VACA-1003	VCA-1003	VVCA-1003
SK-OV-3	89%	53%	V	VACA-1003	VCA-1003	VVCA-1003
SW480	60%	86%	V	VACA-1003	VCA-1003	VVCA-1003
T-47D	51%	94%	V	VACA-1003	VCA-1003	VVCA-1003
T/C-28 a2	90%	80%	V	VACA-1003	VCA-1003	VVCA-1003
T/G HA-VSMC	58%	79%	V	VACA-1003	VCA-1003	VVCA-1003
T2	60%	68%	C	VACA-1004	VCA-1004	VVCA-1004
T84	53%	83%	T	VACA-1002	VCA-1002	VVCA-1002
TF-1	38%	82%	T	VACA-1002	VCA-1002	VVCA-1002
THP-1	47–68%	40–58%	V	VACA-1003	VCA-1003	VVCA-1003
U-2 OS	98%	88%	V	VACA-1003	VCA-1003	VVCA-1003
U-87 MG	43%	91%	T	VACA-1002	VCA-1002	VVCA-1002
U-937	20–30%		C	VACA-1004	VCA-1004	VVCA-1004
U266B1	86%	91%	V	VACA-1003	VCA-1003	VVCA-1003
Vero	79%	97%	V	VACA-1003	VCA-1003	VVCA-1003
WEHI-231	77%	62%	L	VACA-1005	VCA-1005	VVCA-1005
WI-38	75%	91%	R	VACA-1001	VCA-1001	VVCA-1001

Cell Line Optimization Kit for Nucleofector™ II/2b Device

The Cell Line Optimization Nucleofector™ Kit is the ideal tool for the transfection of virtually any difficult-to-transfect cell line with the Nucleofector™ II/2b Device. It enables you to conveniently determine the optimal Nucleofection Condition of your cell line of interest within one experiment. The kit contains two different Cell Line Nucleofector™ Solutions, V and L, each of which should be tested in combination with seven different Nucleofector™ Programs. Fine-tuning for optimal results can then be performed together with our Scientific Support Team.

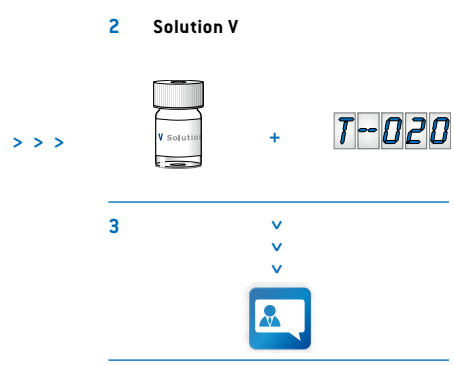
Benefits

- Efficient transfection of virtually any difficult-to-transfect cell line
- Simple and rapid optimization completed within just one experiment

Applications

- Transfection of virtually any difficult-to-transfect cell line with the Nucleofector™ II/2b Device

1 Solution	L	V
Program 1	A-020	A-020
Program 2	T-020	T-020
Program 3	T-030	T-030
Program 4	X-001	X-001
Program 5	X-005	X-005
Program 6	L-029	L-029
Program 7	D-023	D-023



Step 1

The cell line of interest is transfected with the Nucleofector™ Solutions L and V in combination with seven different Nucleofector™ Programs.

Step 2

The Nucleofector™ Solution and Program which result in highest transfection efficiencies with lowest mortality are selected.

Step 3

A further fine tuning of the Nucleofection Conditions can be performed with the help of our Scientific Support Team.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
VCO-1001N	VCO-1001N	Cell Line Optimization Nucleofector™ Kit	100 µL aluminum cuvette	18 reactions

Related Products	Page
Classical Media	108-118

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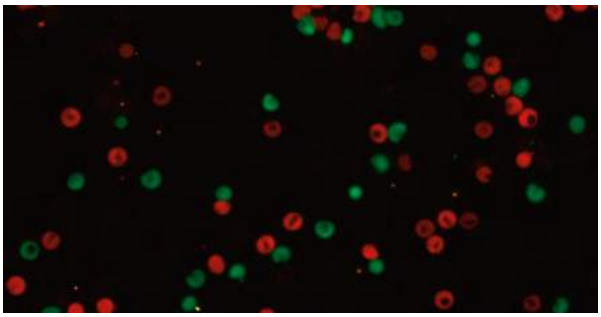
Basic Parasite Nucleofector™ Kits

Parasitic protozoa infect vertebrates and invertebrates and some are even parasitic in plants. In humans, they can cause severe diseases, such as Malaria (*Plasmodium*), Sleeping Sickness (*Trypanosoma*) or *Leishmaniasis* (*Leishmania*). Nucleofection has proven to provide considerably higher transfection efficiencies (e.g., in *Plasmodium berghei and Trypanosoma brucei*) compared to standard methods, such as electroporation or particle bombardment. Due to significant genotypic and phenotypic diversity between species and life cycles, we have developed two Basic Parasite Nucleofector™ Kits (1 and 2) and an easy-to-use Basic Parasite Nucleofector™ Starter Kit.



Nucleofection of the rodent malaria parasite *Plasmodium berghei*. *Plasmodium berghei* parasites were transfected with a reporter vector containing two genes encoding for green fluorescent protein (GFP) and red fluorescent protein (RFP) under control of sex-specific promoters. After selection of transgenic parasites, sexual cells (gametocytes) of these parasites were analyzed by fluorescence microscopy. Male cells showed green and female cells a red fluorescence. (Data kindly provided by Chris Janse, Blandine Franke-Fayard and Andrew Waters, Leiden Malaria Research Group, Department of Parasitology, Leiden University Medical Centre, Netherlands.)

Benefits

– Increased transfection efficiencies compared to standard methods, such as electroporation or particle bombardment

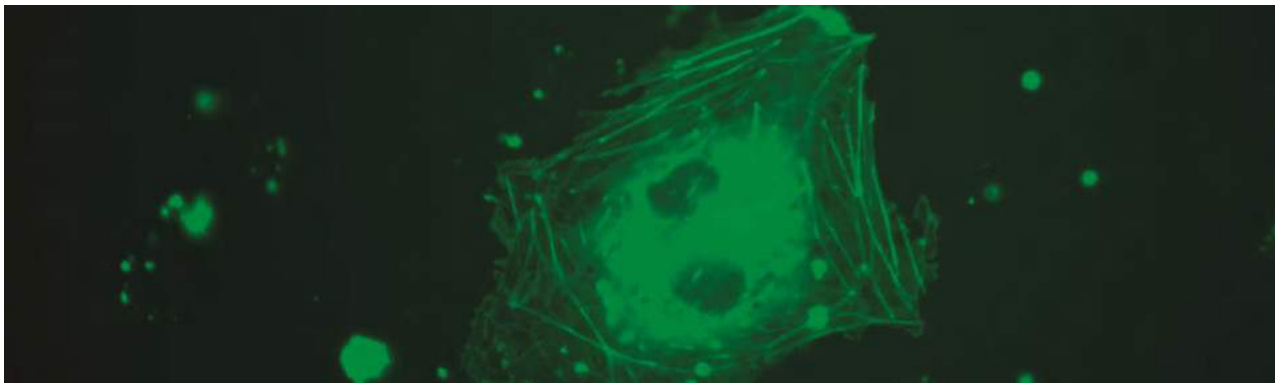
Applications

– Proven results for *Plasmodium berghei and Trypanosoma brucei*

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
VVMI-1011	VVMI-1011	Basic Parasite Nucleofector™ Kit 1	100 µL aluminum cuvette	4 × 25 reactions
VMI-1011	VMI-1011	Basic Parasite Nucleofector™ Kit 1	100 µL aluminum cuvette	25 reactions
VVMI-1021	VVMI-1021	Basic Parasite Nucleofector™ Kit 2	100 µL aluminum cuvette	4 × 25 reactions
VMI-1021	VMI-1021	Basic Parasite Nucleofector™ Kit 2	100 µL aluminum cuvette	25 reactions
VAMI-1011	VAMI-1011	Basic Parasite Nucleofector™ Kit 1	100 µL aluminum cuvette	10 reactions
VAMI-1021	VAMI-1021	Basic Parasite Nucleofector™ Kit 2	100 µL aluminum cuvette	10 reactions
VMI-1001	VMI-1001	Basic Parasite Starter Nucleofector™ Kit	100 µL aluminum cuvette	10 reactions

Nucleofector™ Kit Accessories



Nucleofector™ Kit Accessories

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Nucleofector™ PLUS Supplement	244
Mouse T Cell Nucleofector™ Medium	245
pmaxCloning™ Vector	245

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Introduction

We offer a range of accessory products that can be used in combination with our Nucleofector™ Technology.

- **Nucleofector™ PLUS Supplement** –For cryopreservation of cells in Nucleofector™ Solution
- **Mouse T Cell Nucleofector™ Medium** – For optimal Nucleofection Performance with mouse T cells
- **pmaxCloning™ Vector** – For cloning your gene of interest into a high expression level plasmid

Nucleofector™ PLUS Supplement

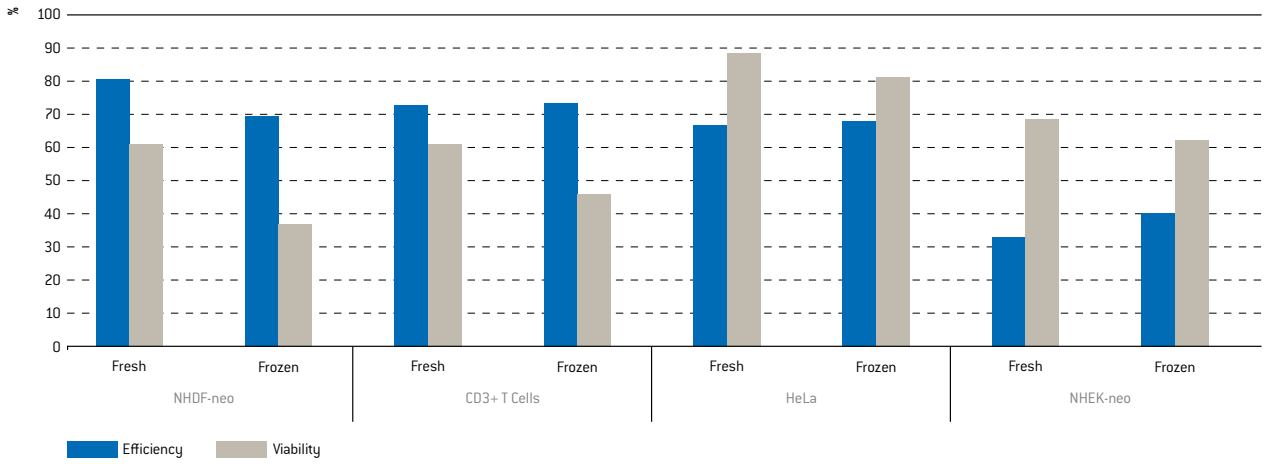
The new Nucleofector™ PLUS Supplement bypass the need of postponing a transfection experiment due to problems with cell culture or primary cell isolation. Moreover it allows for more efficient time-management as freshly isolated or cultured cells do not have to be transfected on the day of isolation or harvesting.
Nucleofector™ PLUS Supplement can be used in conjunction with many existing Nucleofector™ Kits. Just substitute the standard supplement (delivered with regular Nucleofector™ Kit) by Nucleofector™ PLUS Supplement and generate your own cryopreserved ready-to-transfect competent cells.

Benefits

- Minimize variations caused by donor variance, isolation process or cell culture
- More convenient workflow by decoupling of cell isolation and transfection
- Cryopreserved transfection competent cells ready-to-use any time

Applications

- Cryopreservation of primary cells or cell lines in Nucleofector™ Solution
- Transfection experiment series with the same cell batch



Comparison of transfection performance for fresh cells and cells that were cryopreserved in Nucleofector™ PLUS Solution. Data were collected from various experiments to account for variances in cell handling.

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
VS1-00500P	VS1-00500P	Nucleofector™ PLUS-1 Supplement	To determine the supplement required for your Primary Cell Nucleofector™ II/ 2b Kit please refer to the table provided on our website: www.lonza.com/n-plus	500 µL

Mouse T Cell Nucleofector™ Medium

For optimal Nucleofection Performance with mouse T cells it is highly recommended to use Mouse T Cell Nucleofector™ Medium for cell culture steps post Nucleofection.

The medium is included in our Mouse T Cell Nucleofector™ Kit (for the Nucleofector™ II/2b Device), and offered as separate product when using the P3 Kit with the 4D-Nucleofector™ System or the 96-well Shuttle™ Device.

Benefits
- Provides consistent, high-yield Nucleofection Results
- Essential for survival of transfected mouse T cells

Applications
- For use in combination with the P3 Primary Cell 4D-Nucleofector™ or 96-well Nucleofector™ Kits
- For post Nucleofection Culture of mouse T cells

Ordering Information – Medium

Cat. No. NA	Cat. No. EU	Product Name	Size	Storage Conditions
VZB-1001	VZB-1001	Mouse T Cell Nucleofector™ Medium	100 mL	4° to 8°C, do not freeze

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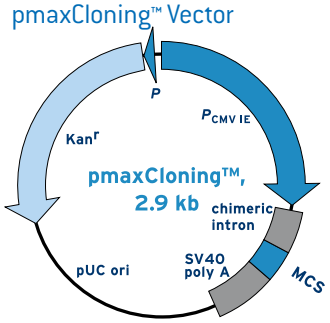
Mouse B Cell Nucleofector™ Kits	190

pmaxCloning™ Vector

Benefits :
- High expression rate in mammalian cells
- License-free use for research purposes
- Multiple cloning site for convenient insertion of the gene-of-interest

NOTE: The CMV promoter is covered under U.S. patent 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA.

pmaxCloning™ Vector



MCS	
Kpn I	Pst I
Pme I	BamH I
Hind III	Xho I
EcoR I	Nhe I
Xba I	Not I
EcoR V	Pme I
	Sac I

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
VDC-1040	VDC-1040	pmaxCloning™ Vector	Concentration: 0.5 µg/µL	20 µg

Notes

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6 Culture and Analysis Tools

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CytoSMART™ System

CytoSMART™ System

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Introduction

With the CytoSMART™ System a new era of personal, affordable live cell imaging begins. The integrated cloud functionality allows cell culture monitoring outside of the lab, i.e. in your office or at home, outside a clean-room or under defined incubator (e.g. hypoxic) conditions. Simply view your cell culture remotely and in real-time in the cloud with any browser-capable system, whether it is your computer, laptop, smartphone or tablet device.

Your Cell Culture is One Click Away

Setting up your CytoSMART™ System requires minimal training and you'll be up and running in minutes. The CytoSMART™ System consists of a mini-microscope roughly the size of a cell culture plate which fits into any incubator. This device is linked to an accompanying tablet which is fixed outside the incubator. Via this tablet you start your CytoSMART™ Projects and capture the images of your cell culture in defined intervals which are then transmitted to the CytoSMART™ Connect Cloud via wireless transmission (Wifi).

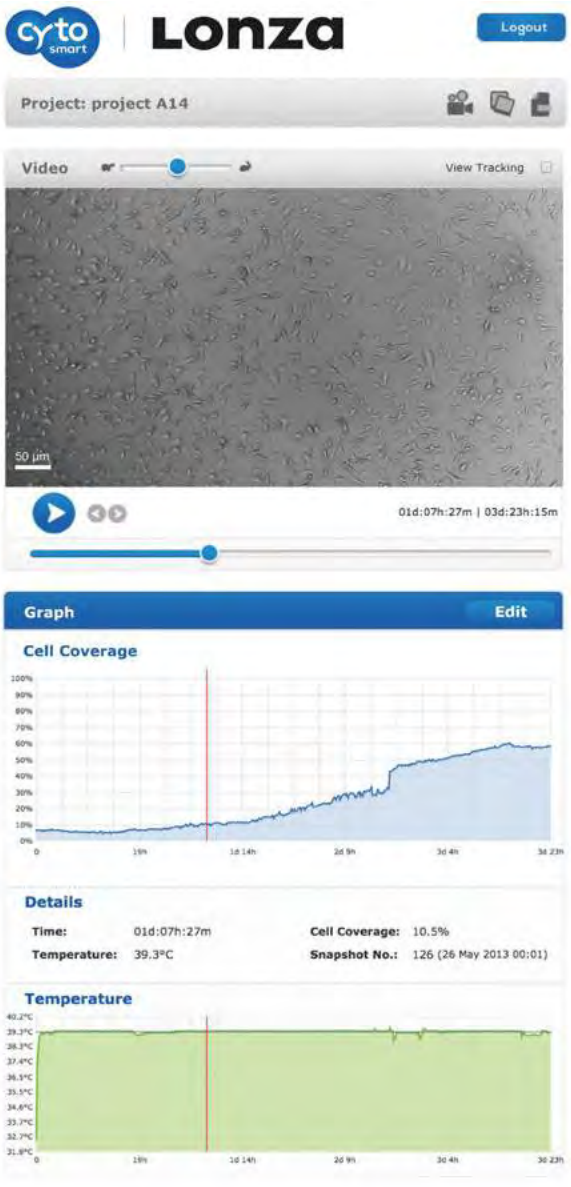
For each CytoSMART™ Project started you will receive an email link which allows you to monitor your cell culture remotely and in real-time with any browser-capable device. All image files are intermediately stored on the tablet assuring full data safety should your WiFi access be interrupted. The CytoSMART™ Connect Cloud is hosted via a cloud hosting service which fulfills German data safety regulations, one of the world's strictest and most secure data storage laws. Images and time-lapse videos of your cell culture can easily be downloaded via the CytoSMART™ Connect Cloud as single images (.jpg files) or as video files (.avi files).

In the absence of a WiFi connection or when data storage on a cloud is not desired, you can also start CytoSMART™ Projects offline, including image taking and video recording. In this case, data files are stored locally on the tablet and can be transferred to your personal computer at a later time.

Benefits

- Easy handling – Set-up of system within minutes
- Small size – Easily fits in every incubator
- Cloud technology – Monitor your cell culture anywhere and anytime
- Low cost – The affordable live cell imaging tool for small budgets

CytoSMART™ System in incubator with technician



CytoSMART™ Connect Project Page as shown on a smart phone.

CytoSMART™ Lux 10X System

The CytoSMART™ Lux 10X System has been specifically designed for applications where viewing a larger cell culture area is required, such as for general cell culture monitoring and documentation or for scratch/migration assays.
The field of view of the CytoSMART™ Lux 10X System is 2.4 x 1.5 mm. The magnification is similar to that typically achieved with a 10x objective using a conventional microscope.

Benefits

- Remote monitoring of your cell culture
- Cell culture standardization through operator independent determination of confluency and automatic alerts for cell culture splitting
- Easy and immediate download of cell culture images or videos from CytoSMART™ Connect Cloud
- Live/dead cell counting

Applications

- Migration assays such as scratch assays
- Documentation of stem cell culture such as reprogramming into iPSC or for mesenchymal stem cells
- Monitoring and documentation of cell culture under restricted conditions such as in clean-rooms or under hypoxic cell culturing conditions
- Determination and documentation of cell growth curves
- Studies to determine influences of various factors on cell culture performance (e.g. compound addition, screening of culture media or growth factors)

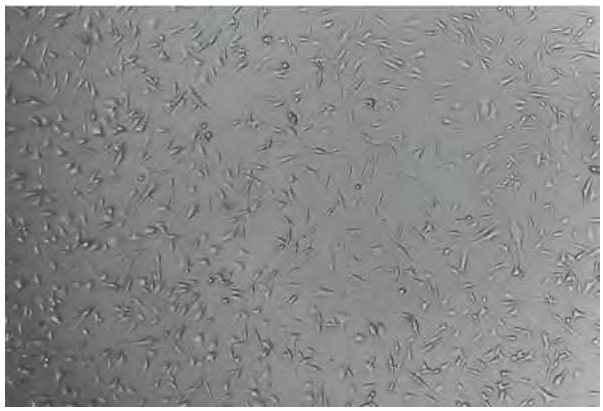
Cell Monitoring and Cell Counting with One Device

The CytoSMART™ System offers a quick and easy-to-use cell counting feature using a standard hemocytometer. Within seconds, the CytoSMART™ App determines the exact number of living cells. Cell counts are reproducible and match the number achieved with manual cell counting.

Cell Counting Applications

- Determine the cell concentration of your suspension cell culture
- Count the number of living cells in your trypan blue stained cell sample in a conventional hemocytometer

CytoSMART™ Lux 10X System with tablet



CytoSMART™ Lux 10X System screenshot of tablet while monitoring C6 glioma cells in incubator.

	A. Cell Type 1			B. Cell Type 2		
	Count 1	Count 2	Count 3	Count 1	Count 2	Count 3
CytoSMART™ Cell Counting [10^6 cells/mL]	2.32	2.28	2.31	1.15	0.9	1.19
Manual Cell Counting** [10^6 cells/mL]	2.24			1.02		

Reproducibility of CytoSMART™ Cell Counting of living cells and comparison with manual cell counting. Cell counting performed with CytoSMART™ Device either counting the same area of the hemocytometer **(A)** or three different areas of a hemocytometer **(B)**.

www.lonza.com/cytosmart

CytoSMART™ Lux 10X System

Continued

Technical Specifications	
Field view	2.4 x 1.5 mm^2
Magnification	100X
Image resolution	1280 x 720 pixels (converted to 640 x 360 on webpage)
Exported formats	JPEG, CSV and AVI
Light source	LED (Green)
Camera	CMOS
Display	Remote tablet touchscreen
Optical filters	No optical filters
Dimensions	133 x 90 x 100 mm (LxWxH)
Weight	0.5 kg
Storage	50 GB online
Power	AC 100-240V, 2A, 10W, 50 / 60Hz
Operating environment	5 to 40°C, 20 – 95% humidity

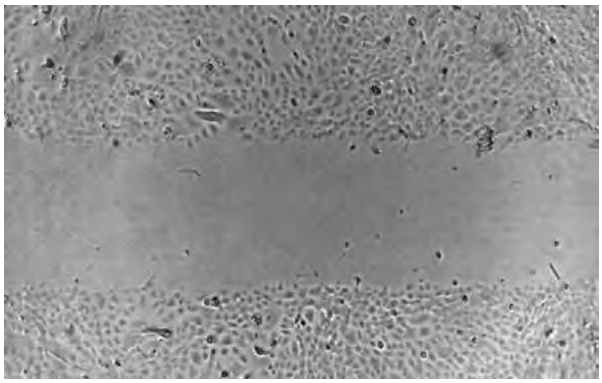


Image of scratch assay cell culture of Normal Human Epidermal Keratinocytes – Adult (192627) at time 0 taken with CytoSMART™ System.

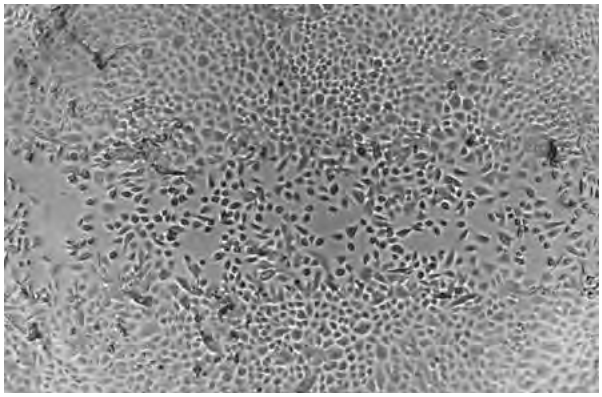


Image of scratch assay cell culture of Normal Human Epidermal Keratinocytes – Adult (192627) at time 8 hours taken with CytoSMART™ System.

Ordering Information – CytoSMART™ System

Cat. No. NA	Cat. No. EU	Product Name	Product Description
AACS-1001	**AACS-1001**	CytoSMART™ Lux 10X System	Contains CytoSMART™ Lux 10X Device, accompanying tablet and two year free CytoSMART™ Connect Cloud Service*
AWCS-1001	**AWCS-1001**	CytoSMART™ Connect Cloud One Year Renewal	Automatic renewal of CytoSMART™ Connect Cloud Service for one year
AAK-2003	**AAK-2003**	CytoSMART™ Stage Attachment	Allows viewing of T162 flasks

Related Products	Page
Primary Cells and Media	53-104

***CytoSMART™ Lux 10X System**

Consists of a CytoSMART™ Lux 10X Device, a tablet and a CytoSMART™ Basic Connect Cloud Service which consists of 1 GB online storage capacity and maximum online storage of any projects started within the previous 7 days (during that time project files such as images and videos can be exported to be saved locally and after that time project files will be deleted from the cloud). During the first two years of CytoSMART™ System ownership, complimentary access is granted to the full CytoSMART™ Connect Cloud Service which offers 50 GB maximum online storage capacity, alert functionality and recording of cell coverage and temperature. If the full CytoSMART™ Connect Cloud Service is not renewed after two years, the system will operate under the basic cloud service. Note that projects started during the initial two-year cloud service will no longer be accessible under the basic cloud service. In this case we recommend storing all necessary images and videos prior to termination of full Connect Cloud Service.

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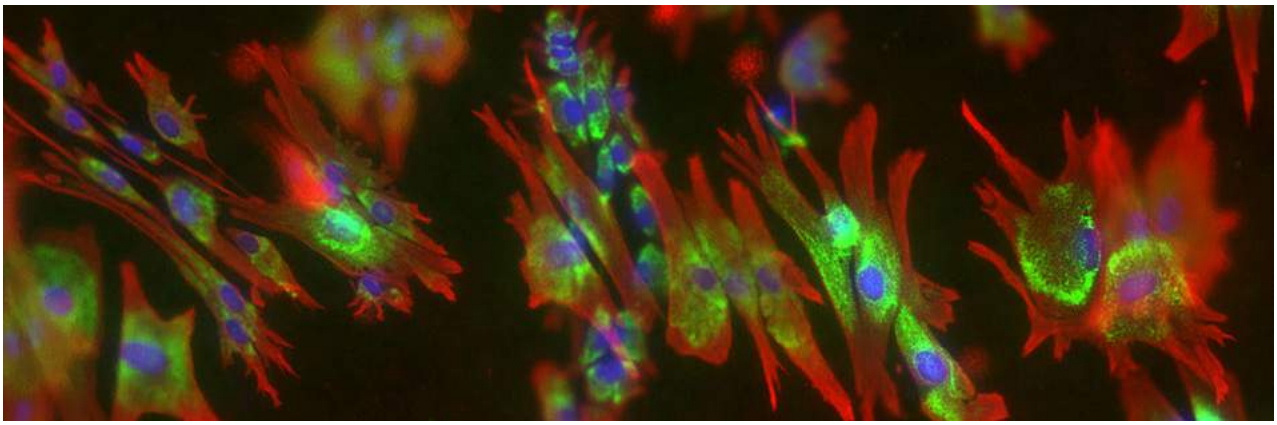
RAFT™ 3D Culture System



RAFT™ 3D Culture System

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Introduction

3D cell culture differs significantly from the traditional 2D culture that most researchers have been using for decades. There is a trending shift both in academia and industry to personalized research solutions and more *in vivo* like models to understand cell behavior. This is fueling the growing market need for better solutions in 3D cell culture. RAFT™ 3D Cell Culture System does just that and fills the growing demand for our customers.

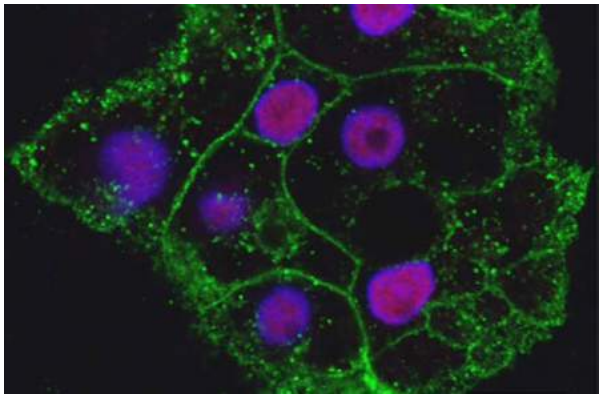
What are the Benefits of the RAFT™ System over 2D or Other 3D Methods?

An important differentiation of the RAFT™ System versus other platforms or 2D culture methods is its versatility. This is especially important for certain applications such as development of *in vitro* liver fibrosis models or corneal models. With the RAFT™ System, cells can be cultured within a high-density collagen scaffold, or on top, or both. The addition of permeable membrane cell culture inserts provides other extensions to the system allowing the generation of barrier models including air-lift models. This approach allows for the formation of complex *in vitro* models and facilitates an improved understanding of cellular growth, differentiation and cell-to-cell interactions. Furthermore, the system is compatible with a variety of cell types and has already been used to successfully generate 3D cultures in a variety of research areas including oncology, toxicology, barrier modeling and pulmonary research.

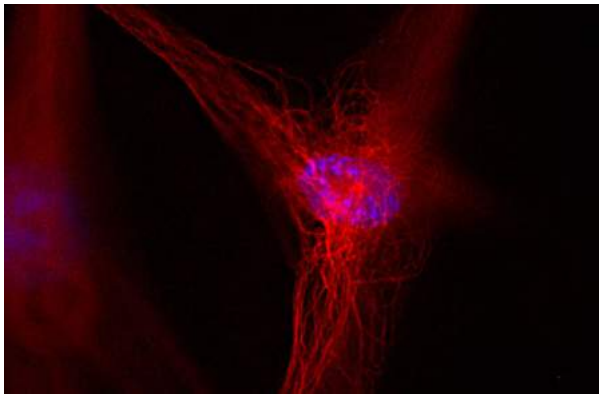
How Does the RAFT™ System Work?

The RAFT™ System has been designed with usability in mind, with simple easy-to-follow protocols that allow researchers to set up 3D cell cultures in less than an hour. The RAFT™ Kit includes a collagen type I solution and biocompatible absorbers, which remove the medium from cell-seeded collagen hydrogels and allow researchers to control both cell concentration and matrix density. The versatile RAFT™ Kit is available in 96 or 24-well formats and is suitable for analysis using a wide variety of imaging, biochemical and histological techniques.

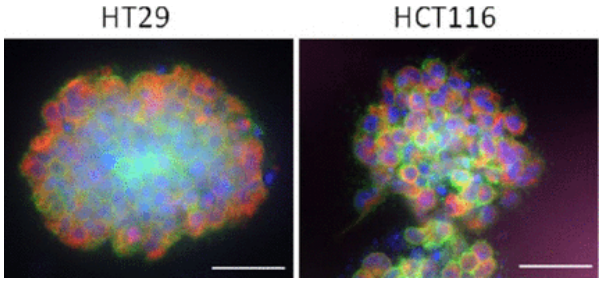
In combination with Lonza's human primary cells and media solutions, the RAFT™ 3D Cell Culture System empowers researchers to create biologically relevant cell culture models for use across drug discovery and research applications.



iPSC-derived hepatocytes in RAFT™ System form 3D canalicular structures and exhibit cell polarisation. Data courtesy of Gieseck *et al.*, 2014, PLOSOne.



Dermal fibroblasts fixed and stained after 11 days in RAFT™ System.



Colon cancer cell lines form "tumoroids" in RAFT™ 3D Cell Culture System.

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What Does the RAFT™ System Consist of?

- **RAFT™ Reagent Kit** – Consists of rat tail collagen 1 as the extracellular matrix scaffold and a specially-designed neutralizing solution that enables neutralization to be carried out in a single step instead of using an error-prone and tedious titration method. The kit also contains 10X MEM to support cells through the RAFT™ Process.
- **Novel RAFT™ Absorbers** – The biocompatible absorbers are designed to work with standard 96- or 24-well cell culture plates as well as 24-well cell culture inserts. Once the cells are mixed with RAFT™ Reagents, the absorbers are utilized to compress and absorb excess liquid from the collagen mixture. This results in the creation of well-controlled, high-density collagen scaffolds embedded with cell type(s) of choice. The RAFT™ System is one of the few commercially available kits that allows users to create high-density collagen scaffolds that are representative of a natural, more in-vivo like environment for cells to grow and interact more efficiently.

RAFT™ 24-well Insert Kit for Cell-Culture Inserts (catalog number 016-1R25 and 016-1R33) – RAFT™ Insert Absorbers are designed to be used with 24-well permeable membrane cell culture inserts. This additional kit is offered to enhance the versatility of the system further by allowing researchers to generate co-culture models, and barrier models including air-lift models. Please note no cell culture inserts are supplied within the RAFT™ 24-well insert kit due to variation in pore size. Please contact Lonza's Scientific Support Team for a detailed list of RAFT™ Kit compatible inserts or visit our frequently asked questions section.

www.lonza.com/faq

RAFT™ 3D Culture System consists of RAFT™ Reagent Kit and Absorbers for 96-well, 24-well or trans-well inserts.

Absorbers being utilized with transwell inserts in a 24-well plate

3D Cultures created with the RAFT™ 3D Cell Culture System. Cells are embedded in collagen and the scaffold is condensed into thin, high-density 3D cultures for tissue modeling and long-term culture maintenance.

RAFT™ 3D Cell Culture System

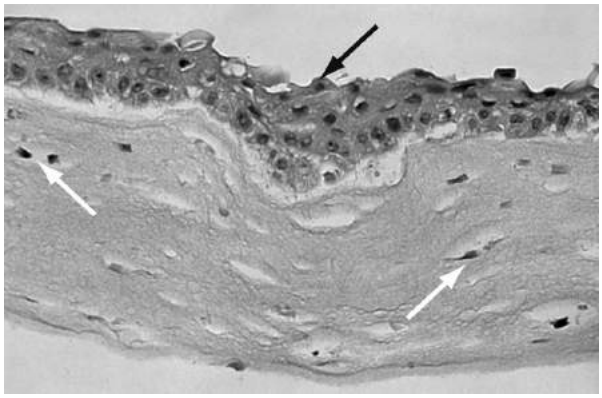
Benefits

- Create 3D cultures using a user-friendly system with optimized protocols
- Robust 3D Cultures that are 120 µm thick enabling easy handling and long term culture
- Flexible formats – 96-well, 24-well plate, or 24-well cell-culture inserts
- Scale up and automation - used with leading manufacturers' products such as Freedom EVO® Liquid handling and Cytation™ 3.

Applications

RAFT™ 3D Cultures have been tested in the following application areas but can easily be applied to broader research areas

- Cancer Research
- Toxicology
- Dermal Research
- Corneal Models
- Blood Brain Barrier Models
- Pulmonary research



A 3D *in vitro* human cornea model containing biomimetic corneal limbal crypts established in RAFT™ System. H&E stained paraffin embedded section shows that the HLE (human limbal epithelial) cells formed a healthy, 3-4 cell multi-layered epithelium on the flat surface of the HLF (human limbal fibroblasts) embedded in collagen. Data Courtesy of *Levis et. al. (2013)* Biomaterials.

RAFT™ Sequence (A)Empty well or insert, (B) Cell and collagen mix forms hydrogel at 37°C, (C) Absorber contacts the hydrogel, (D) Liquid starts to be absorbed, (E) Process concentrates cells and collagen, (F) In less than 1 hour culture formation complete.

Visit our web site and watch a 2 minute video on how the RAFT™ Sytem works.
www.lonza.com/raft

Ordering Information – RAFT™ 3D Cell Culture System

Cat. No. NA	Cat. No. EU	Product Name	Product Description
016-1R10	016-1R10	RAFT™ 96-well Bundle Kit	Contains (016-0R94 & 016-0R92)
016-1R24	016-1R24	RAFT™ 24-well Bundle Kit	Contains (016-0R94 & 016-1R32)
016-1R25	016-1R25	RAFT™ 24-well Insert Bundle Kit	Contains (016-0R94 & 016-1R33)
016-1R16	016-1R16	RAFT™ Small Kit	12 rxns, reagent & 24-well plate absorbers
016-1R32	016-1R32	RAFT™ Absorbers	Absorbers for 24-well Plate, 48 qty
016-1R33	016-1R33	RAFT™ Insert Absorbers	Absorbers for 24-well Plate, 48 qty, for Inserts
016-0R92	016-0R92	RAFT™ Plate Kit	4 x 96-well clear plate and 4 x 96-well absorber
016-0R94	016-0R94	RAFT™ Reagent Kit	Reagent Kit for 3D Cell Cultures

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BioAssay Products and Services

Bioluminescent Cell Health

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Bioluminescent Cell Health

Bioluminescent Cell Health

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ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit	263

Introduction

Achieve outstanding sensitivity when evaluating cell proliferation and cell death with our easy-to-use bioluminescent cell health assays. These assays are suitable for use with adherent or suspension cultures of cell lines and primary cells.

ViaLight™ Cell Proliferation and Cytotoxicity BioAssay Kits are designed to provide unprecedented speed and sensitivity for cytotoxicity and cell proliferation studies, and are safer than traditional radioactive methods.

The ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit is a bioluminescent, non-destructive cytolysis assay kit designed to measure the release of the enzyme adenylate kinase (AK) from damaged cells.

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

ViaLight™ Cell Proliferation and Cytotoxicity BioAssay Kit

ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay Kits provide unprecedented speed and sensitivity for cytotoxicity and cell proliferation studies. Ideal for 3D and adherent cell cultures, safer than traditional radioactive methods, ViaLight™ protocols are as fast and easy as other viability kits without any shaking needed. The ViaLight™ Kit incorporates bioluminescent detection of cellular ATP as a measure of viability. It delivers high, stable luminescent signals for an extended period of time, providing greater experimental design flexibility. The easy, two-step, homogeneous assays are scalable for high-throughput applications in both 96- and 384-well formats on a variety of luminometers or scintillation counters.

The ViaLight™ MDA Plus Microbial Proliferation and Cytotoxicity Kit has been optimized for use with bacteria or yeast. The basic reaction remains the same as the ViaLight™ Plus Kit, however, the lysis reagent has been optimized for bacteria and yeast. Sensitivity is 1,000 bacterial cells or 100 yeast cells per well.

Benefits

- **Fast** – Results from a 96-well and 384-well plates can be processed and analyzed in <15 minutes
- **Sensitive** – Detect as few as ten cells allowing for lower seeding densities and more assays
- **Convenient** – Simply add two reagents directly to your culture well and read
- **Robust** – Dynamic range of five decades with both adherent or suspension cultures

Applications

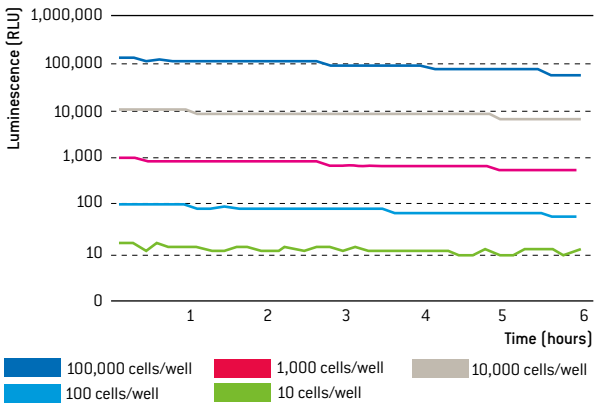
- Cell proliferation studies
- Cytotoxicity studies
- Cell viability studies
- High-throughput screening

Specifications	
Detection limit:	ViaLight™ Plus – ten mammalian cells; ViaLight™ MDA – 1,000 bacteria per well
Assay time:	1 second integrated reading per sample <15 minutes per 96-well plate
Linear range:	Greater than five orders of magnitude
Reproducibility:	Typical coefficient of variation (CV) ≈6%
Correlation:	Excellent with tritiated thymidine (typically, R^2=0.995, p<0.01)
Suitable cell types:	Mammalian cells (adherent and non-adherent); bacterial and yeast cells

2°C to 8°C, do not freeze prior to reconstitution

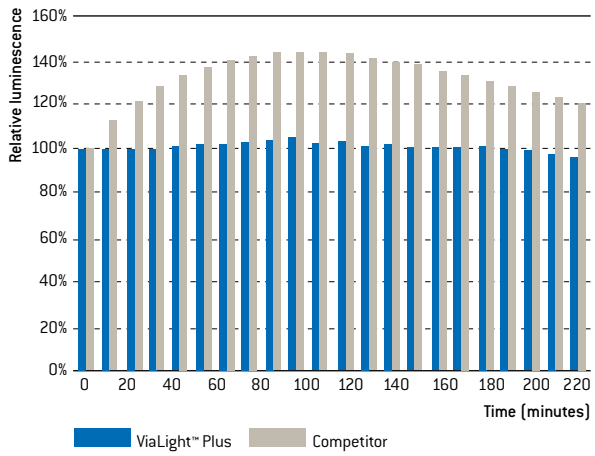
www.lonza.com/vialight

ViaLight™ Plus BioAssay Kit Signal Stability



Extended luminescent signal stability (half life >6 hours) regardless of the number of cells used facilitates batch processing and ensures consistent results.

Comparison of Signal Stability Over Time



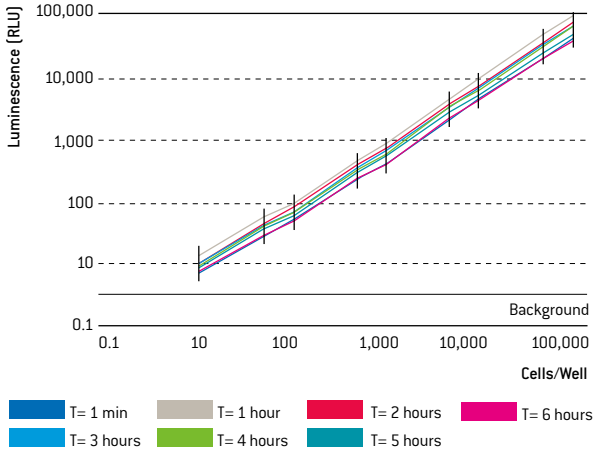
Light output with ViaLight™ Plus BioAssay Kit and a competitive luminescent assay kit with A549 cells was compared over time. The consistent light output, 10-fold lower background and exceptional lysis capabilities of the ViaLight™ BioAssay Kit ensure superior results.

Ordering information on the next page.

ViaLight™ Cell Proliferation and Cytotoxicity BioAssay Kits

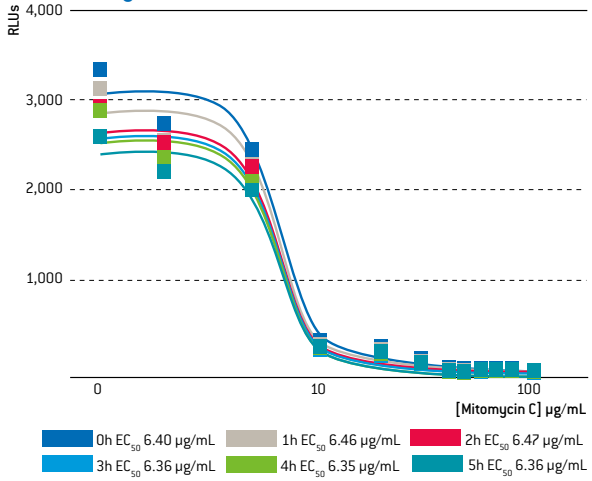
Continued

ViaLight™ Plus BioAssay Kit Sensitivity and Extended Linearity



Light output at various times after reagent addition and with increasing numbers of K562 cells demonstrate the exceptional sensitivity and dynamic range delivered by the ViaLight™ Plus BioAssay Kit.

EC 50 Data Generated Using ViaLight Plus Shows Consistency Over Time



HepG2 cells were incubated with the alkylating agent Mitomycin C for 48 hours and then assayed using ViaLight™ Plus. The experiemental values are the mean of eight replicate samples read every hour over a 5 hour period. The EC values remain consistent over the 5 hour read period.

Ordering Information – BioAssay Kit

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
LT07-322	LT07-322	ViaLight™ MDA Plus Microbial Proliferation and Cytotoxicity BioAssay Kit		2°C to 8°C, do not freeze*	10,000 tests
LT07-122	LT07-122	ViaLight™ MDA Plus Microbial Proliferation and Cytotoxicity Kit		2°C to 8°C, do not freeze*	1,000 tests
LT17-221	LT17-221	ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay Kit	With 5 white TC plates	2°C to 8°C, do not freeze*	500 tests
LT07-321	LT07-321	ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay Kit		2°C to 8°C, do not freeze*	10,000 tests
LT07-221	LT07-221	ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay Kit		2°C to 8°C, do not freeze*	500 tests
LT07-121	LT07-121	ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay Kit		2°C to 8°C, do not freeze*	1,000 tests

*Product may be stored frozen after reconstitution

Related Products	Page
RAFT™ 3D Cell Culture System	250
ATP Standard	276
Clear Bottom, White Walled TC Plates	276
ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit	263

ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit

The ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit is a bioluminescent, non-destructive cytolysis assay kit designed to measure the release of the enzyme adenylate kinase (AK) from damaged cells. The enzyme actively phosphorylates ADP to form ATP and the resulting ATP is measured using a bioluminescent luciferase reaction. As the level of cytolysis increases, the amount of AK in the supernatant also increases, which results in emission of higher light intensity by the ToxiLight™ Reagent. There is no need for cell lysis; measurements can be taken directly from the supernatant.

Benefits

- **Highly sensitive** – Detect as few as ten cells
- **Non-destructive** – Eliminates the need to lyse cells, allowing multiple tests on the same sample
- **Simple** – Addition of a single reagent directly to cells or supernatant aliquot
- **Fast** – Results from a 96-well 384-well plates can be processed and analyzed in <10 minutes
- **Flexible** – Supernatants can be frozen for long term studies with no loss of AK activity

Applications

- Cytotoxicity studies
- High content screening
- Combination assays

2°C to 8°C, do not freeze prior to reconstitution

www.lonza.com/toxilight

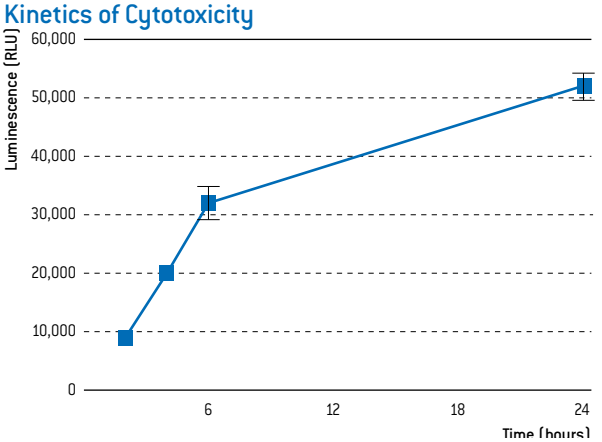
Specifications	
Number of tests per kit:	500x (5 × 96-well pl ates) 1,000x (10 × 96-well plates) 10,000x (100 × 96-well plates)
Detection limit:	10 dead cells per well in homogeneous mode and 50 dead cells per well when the supernatant is sampled from the wells
Assay time:	1 second integrated reading per sample <15 minutes per 96-well plate
Suitable cell types:	All mammalian cells, adherent and non-adherent
Operating temperature:	Ambient
Linear range:	Greater than three orders of magnitude
Reproducibility:	Typical coefficient of variation (CV) ≈5%
Correlation:	Shows excellent correlation with other membrane permeability assays such as propidium iodide
Recommended equipment:	Microplate luminometer with or without reagent injectors. Microplate liquid scintillation counter with luminescence (i.e. out of coincidence) mode

Ordering information on the next page.

ToxiLight™ Non-destructive Cytotoxicity BioAssay Kit

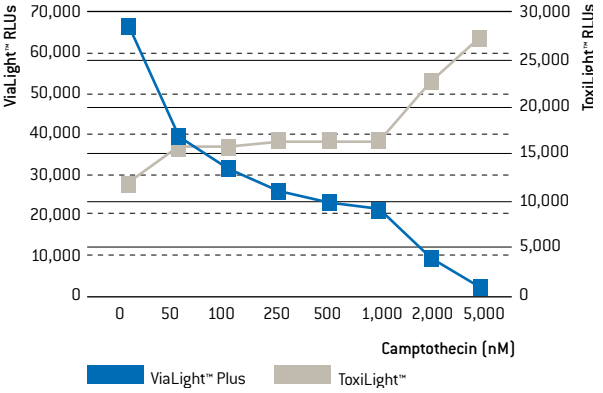
Continued

Kinetics of Cytotoxicity



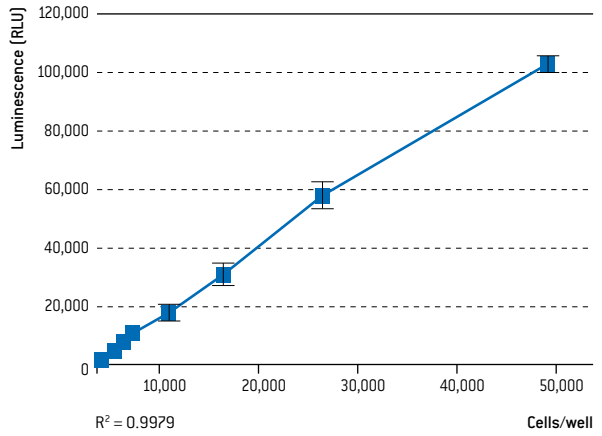
Samples (20 µL) of culture supernatant from cells treated with camptothecin were collected at various times and assayed for cytotoxicity.

Identify Dose-dependent Activities in Cells



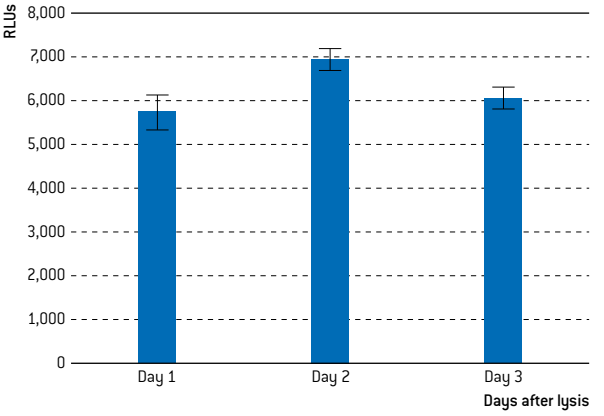
Comparison of ViaLight™ Plus and ToxiLight™ Kits using HUVECs dosed with camptothecin. The ATP levels indicated by the ViaLight™ Plus RLUs reduce steadily in a dose-dependent manner. At the lower drug doses, the AK released from the cells is relatively low compared with that of the control, only increasing dramatically at the highest drug doses.

ToxiLight™ BioAssay Kit is Sensitive Down to Ten Cells



Exceptional sensitivity and wide dynamic range results in exceptional experimental flexibility.

Adenylate Kinase is Stable Over Three Days



Jurkat cells were seeded at 10^5 cells/mL and immediately lysed using the ToxiLight™ 100% Lysis Reagent. The stability of the released AK was measured at 24 hours, 48 hours, and 72 hours after release with no significant loss in activity.

Ordering Information – BioAssay Kit

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
LT17-217	LT17-217	ToxiLight™ Non-Destructive Cytotoxicity BioAssay Kit	With 5 white TC plates	2°C to 8°C, do not freeze*	500 tests
LT07-117	LT07-117	ToxiLight™ Non-Destructive Cytotoxicity BioAssay Kit		2°C to 8°C, do not freeze*	1,000 tests
LT07-217	LT07-217	ToxiLight™ Non-Destructive Cytotoxicity BioAssay Kit		2°C to 8°C, do not freeze*	500 tests
LT07-517	LT07-517	ToxiLight™ 100% Lysis Control Set	Sold separately	2°C to 8°C, do not freeze	200 tests (10 mL)

*Product may be stored frozen after reconstitution

Related Products	Page
RAFT™ 3D Cell Culture System	250
ViaLight™ Plus, 500 test kit	262
Clear Bottom, White Walled TC Plates	276

Cell Function

Cell Function

Introduction	266
PDELight™ HTS cAMP Phosphodiesterase Assay Kit	267
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Introduction

The first group of cell function assays is mainly applied in high-throughput screening environments. They are homogeneous, high sensitivity, luminescence-based assays for enzyme targets including phosphodiesterases (PDELight™) and cylases (PPiLight™).

The second group of cell function assays measure the specific activities of different cell types including cell lines and primary cells. Adipocytes, MSCs, and ADSCs can have lipid metabolism measured with AdipoRed™ and AdipoLyze™. Bone cells like osteoclasts and osteoblasts, and even differentiated MSCs and ADSCs can have bone remodeling measured with OsteoAssay™, OsteoLyse™ and OsteoImage™.

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PDELight™ HTS cAMP Phosphodiesterase Assay Kit

The PDELight™ HTS cAMP Phosphodiesterase Assay Kit is a generic, homogeneous assay designed for use in high-throughput screening to identify inhibitors of phosphodiesterase activity and IC_{50} determinations. The assay utilizes a robust and highly sensitive luciferase-based bioluminescent system to quantify the AMP produced from the hydrolysis of cyclic AMP by phosphodiesterases. AMP is directly converted to ATP and quantitated as light, with nearly a photon of light emitted for every molecule of ATP produced. The assay is sensitive, robust and reproducible. Unlike other phosphodiesterase assays, the PDELight™ Kit does not require costly radioactivity, the use of beads, modified substrates, or antibodies.

Benefits

- **Simple** – Only one reagent to add
- **Generic platform** – The same assay can be used for all cAMP dependent phosphodiesterases
- **Rapid assay** – Complete a 384-well plate in <3 minutes
- **Sensitivity** – Allows for the use of enzyme in 96- or 384-well format
- **Reproducible and robust** – Typical Z´ values >0.8, with good, clean hits

Applications

- cAMP dependent phosphodiesterase activity screening
- IC_{50} determinations

Specifications

- AMP range: <10 nM–20 µM
- Phosphodiesterases: cAMP phosphodiesterases
- Reproducibility: Typical coefficient of variation (CV) is <5%. Typical Z´ value >0.8
- Assay time: <3 minutes per plate

2°C to 8°C, do not freeze prior to reconstitution

PDELight™ Kit Protocol

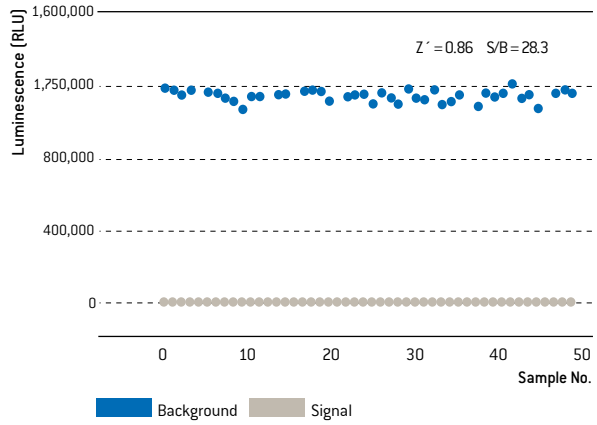
10 µL of inhibitor (40 µM in 10% DMSO)
+
10 µL phosphodiesterase
+
20 µL of cAMP substrate (40 µM)
(Incubate for 30–60 minutes at room temperature)
+
20 µL of PDELight™ Detection Reagent
(Incubate for 10 minutes at room temperature)
▼
Measure luminescence
(0.1–1 second/well)

Ordering information on the next page.

PDELight™ HTS cAMP Phosphodiesterase Assay Kit

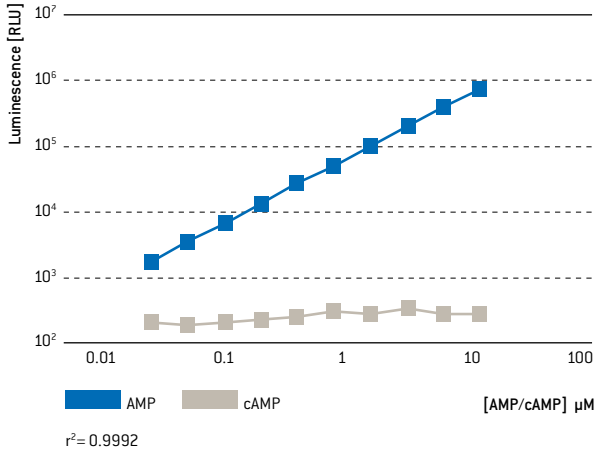
Continued

Reproducible, High Signal to Background Ratios



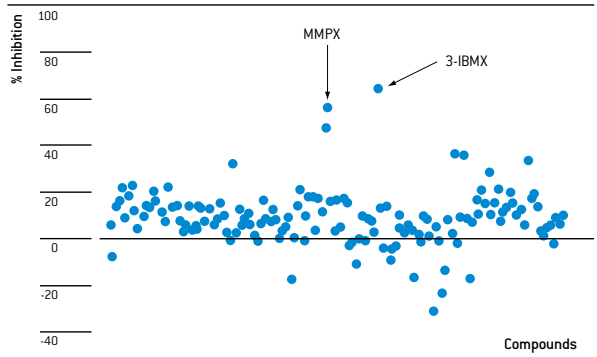
Signal and background determinations were assessed using a single phosphodiesterase demonstrating typical high quality data. Z´ results are typically greater than 0.8 with excellent signal to background ratios.

Linear Detection of AMP

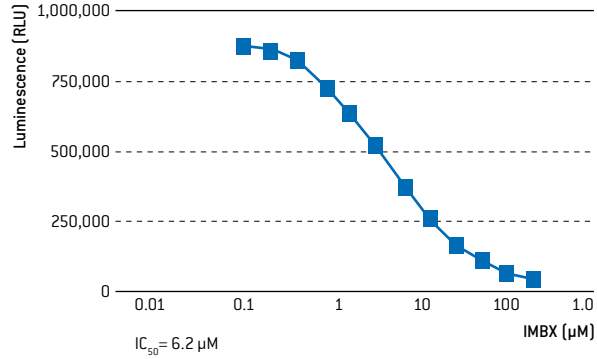


The PDELight™ Kit measures the AMP produced as a result of phosphodiesterase activity. The PDELight™ Detection Reagent measures AMP up to 20 µM. The PDELight™ Detection Reagent is specific for AMP and not cAMP.

Rapid Screening and IC_{50} Determinations with the PDELight™ Assay Kit



Left Image: A library containing 150 pharmacologically active compounds was screened using the PDELight™ Kit. The compounds MMPX and 3-IBMX were identified as inhibiting phosphodiesterase activity greater than 50% at 10 µM.



Right Image: An IC_{50} of 6.2 µM was determined for 3-IBMX.

Ordering Information – Assays and Reagents

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
LT07-600	LT07-600	PDELight™ HTS cAMP Phosphodiesterase Assay Kit	2°C to 8°C, do not freeze*	500 tests

*Product may be stored frozen after reconstitution

PPiLight™ Inorganic Pyrophosphate Assay

Inorganic pyrophosphate (also called diphosphate, pyrophosphoric acid or PPi) is a small diphosphate molecule that is required as a substrate for the product formed from a number of different enzymatic reactions. Enzymes that utilize PPi as a substrate may include phosphotransferases and pyrophosphatases. Enzymes that generate PPi are more numerous and may include cyclases, hydrolases and ligases.

The PPiLight™ Inorganic Pyrophosphate Assay is a non-radioactive bioluminescent assay for the detection of inorganic pyrophosphate. In the presence of PPi, the detection reagent catalyses the conversion of AMP to ATP. The assay uses luciferase, which produces light from the newly formed ATP and luciferin.

Benefits

- **Fast** – Measure enzyme activity via pyrophosphate consumption in 1 hour
- **Simple** – Easy two-step luminescent assay with no radioactive substrates, beads or antibodies required
- **Wide detection range** – Linear range from 0.02 µM to 10 µM
- **Sensitive** – Sensitive to 0.02 µM
- **Versatile** – Scalable to 96- and 384-well formats

Applications

- Measure activity of phosphotransferases and pyrophosphatases
- Measure activity of cyclases, ligases, hydrolases and DNA polymerases

Specifications

- Number of tests: 500 tests in 96- or 384-well plates
- PPi range: 0.02 µM–10 µM in a 100 µL sample
- Assay time: 1 hour
- Operating temperature: Ambient
- Reproducibility: r^2 value >0.95

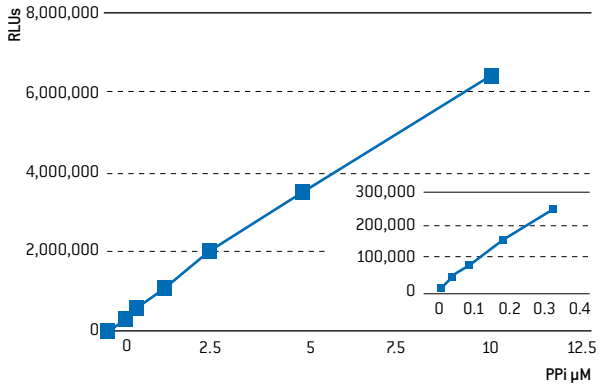
2°C to 8°C, do not freeze prior to reconstitution

Ordering information on the next page.

PPiLight™ Inorganic Pyrophosphate Assay

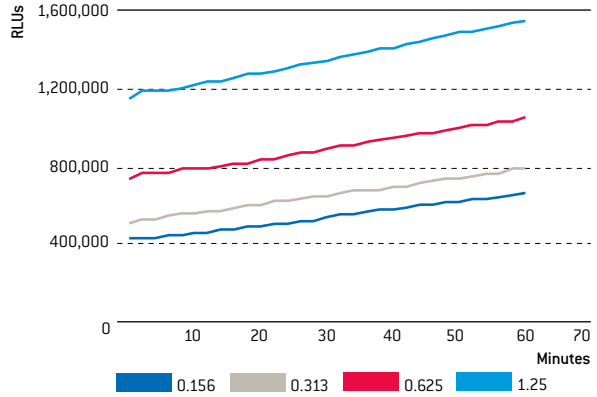
Continued

Light Produced Directly Proportional to PPi Present



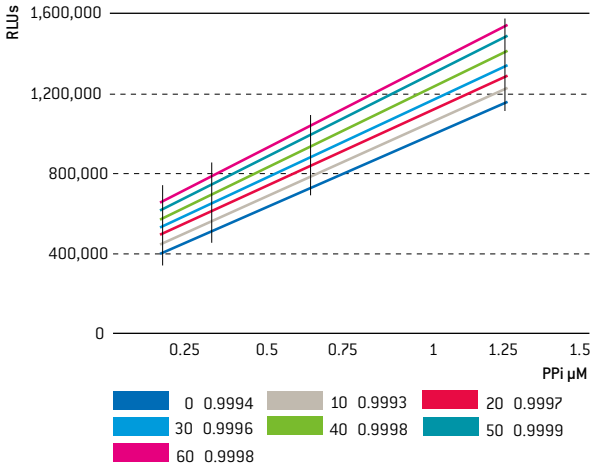
Typical linear PPi standard curve using the PPiLight™ Inorganic Pyrophosphate Assay. Sensitivity is typically 0.02 µM to 10 µM with r^2 values >0.95.

Light Increases Proportionally to PPi Concentration



PPi signal increases over time at a steady, proportional rate as PPi concentration increases. Signal linearity is constant throughout a 1 hour incubation.

Linear signal



The linearity of the signal generated with varying concentrations of PPi was assessed over 1 hour. Linearity is not affected by PPi concentration increase.

Ordering Information – Assays and Reagents

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
LT07-610	LT07-610	PPiLight™ Inorganic Pyrophosphate Assay	2°C to 8°C, do not freeze*	500 tests

*Product may be stored frozen after reconstitution

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

AdipoRed™ Assay Reagent

Quantify Intracellular Lipid Accumulation

The AdipoRed™ Assay Reagent is designed for assessing the effect of compounds on the differentiation of preadipocytes or on lipid utilization in mature adipocytes. The lipophilic AdipoRed™ Assay Reagent specifically partitions into the fat droplets of differentiated adipocytes and fluoresces at 572 nm.

This objective, high-throughput, homogeneous, fluorescence-based assay quantifies the accumulation of intracellular triglycerides and provides significant advantages to drug discovery efforts in the field of obesity and diabetes research. It is more sensitive than other methods, such as the Oil Red O assay, and is much faster and easier than Northern and Western blots.

Benefits

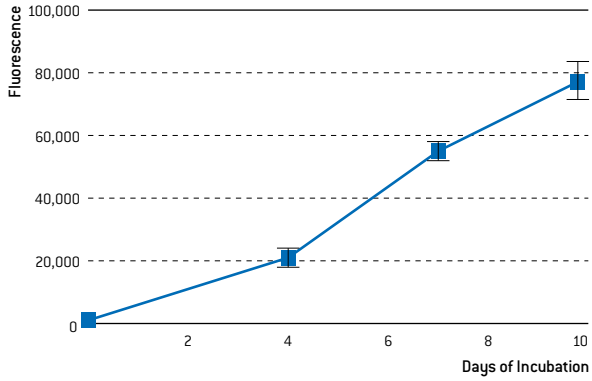
- **Convenient** – Simply replace cell culture medium with PBS, add AdipoRed™ Reagent and read in a standard fluorimeter
- **Fast** – Process an entire 96-well plate in as little as 20 minutes
- **Effective** – Provides objective, high-throughput measurement of the accumulation of intracellular triglycerides, with high signal-to-noise ratios

Applications

- Differentiation of preadipocytes
- Lipid utilization

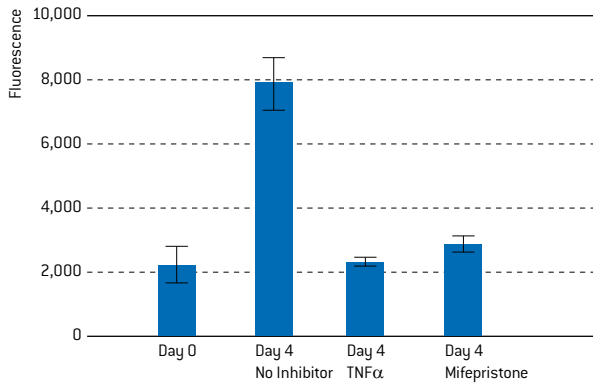
Room temperature (15°C to 30°C)

Quantitation of Intracellular Triglyceride Accumulation



Poietics™ Primary Human Preadipocytes were induced to differentiate and assayed using the AdipoRed™ Assay Reagent.

Inhibition of Adipocyte Differentiation Assayed with AdipoRed™ Assay Reagent



Poietics™ Primary Human Preadipocytes were induced to differentiate in the presence of TNFα, Mifepristone or no inhibitor. Lipid accumulation was assayed after 4 days in culture.

Ordering Information – Assays and Reagents

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
PT-7009	PT-7009	AdipoRed™ Assay Reagent	15°C to 30°C	5 × 4 mL

Related Products	Page
AdipoLyze™ Lipolysis Detection Assay	272
Adipose Derived Stem Cells	19
Human Mesenchymal Stem Cells	29
Human Subcutaneous and Visceral Preadipocyte Cells	27
PGM™ 2 Preadipocyte Growth Medium-2 BulletKit™	28

AdipoLyze™ Lipolysis Detection Assay

AdipoLyze™ Lipolysis Detection Assay is a fast, sensitive, fluorescent *in vitro* assay used for detecting small quantities of glycerol in cells undergoing lipolysis. The AdipoLyze™ Lipolysis Detection Assay can quantitate *in vitro* lipolysis of adipogenic cell lines and primary cells of both subcutaneous and visceral origin.

A unique advantage of this assay is the ability to accurately detect very low levels (down to 0.44 µM or 0.04 µg/mL) of glycerol due to the specificity of the enzymes and the dynamic range of the recommended standard curve.

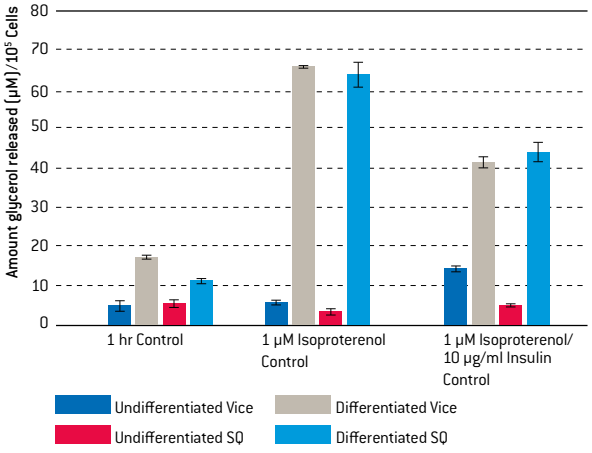
Benefits

- Quantatively measures glycerol, a product of the process of lipolysis
- Completed in <2 hours
- Sensitive enough to detect low levels of glycerol (0.44 µM or 0.04 µg/mL)
- For use in 96-well plate format, but scalable to 384-well

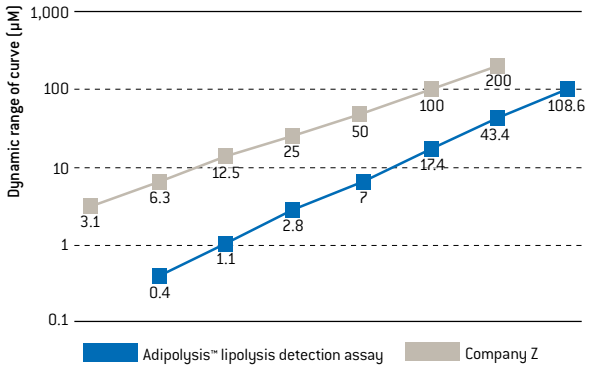
Applications

- Lipolysis detection
- Fat metabolism

-20°C



Poietics™ Human Subcutaneous and Visceral Preadipocyte Cells (undifferentiated and differentiated) were incubated with 1 µM isoproterenol with or without insulin (10 µg/mL) for 1–3 hours and compared to a control culture. Glycerol release was measured using the AdipoLyze™ Lipolysis Detection Assay on a 96-well plate reader.



Comparison of glycerol standard curve dynamic range and sensitivity for Company Z's curve and AdipoLyze™ Lipolysis Detection Assay curve. Lona's standard curve detects glycerol at levels 3.7 times lower than the competition to allow users to discern subtle changes in their cells.

Ordering Information – Assays and Reagents

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
193339	193339	AdipoLyze™ Lipolysis Detection Assay	-20°C	96-wells

Related Products	Page
AdipoRed™ Assay Reagent	271
Human Mesenchymal Stem Cells	29
Human Subcutaneous and Visceral Preadipocyte Cells	27
PGM™ 2 Preadipocyte Growth Medium-2 BulletKit™	28

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OsteoAssay™ Human Bone Plate

Measure Osteoclastic Bone Resorption

The OsteoAssay™ Human Bone Plate provides a thin layer of adherent human bone for the culture of primary human or non-human osteoclasts, osteoclast precursors, and immortalized cell lines. Cells can be stained with standard cytochemical (e.g., TRAP) or immunofluorescent techniques. Assays for measuring bone resorption and/or enzyme activity can be performed easily by sampling the cell culture supernatant.

Benefits

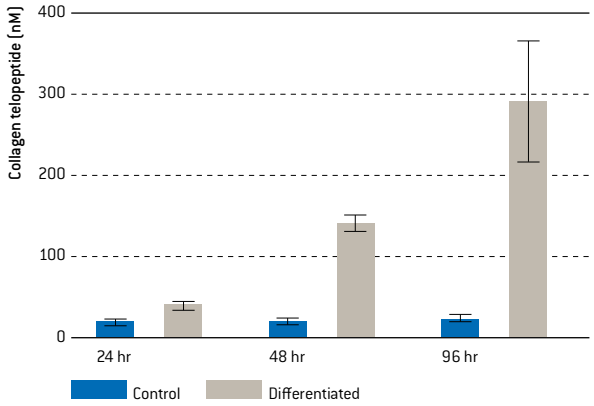
- **Convenient** – Ready-to-use plates with human bone chips attached to wells eliminates the need for dentine or animal bone slices
- **Simple** – Cells can be seeded onto the surface of the OsteoAssay™ Plate using traditional cell culture protocols
- **Flexible** – Can be used with a variety of cell types and cell-based assays
- **Novel** – Contains real human bone for more biologically relevant results

Applications

- Bone resorption
- Osteoclast precursor differentiation
- Osteoclast enzymatic activity

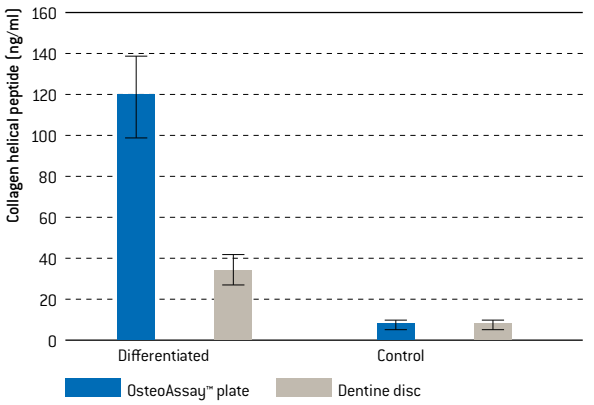
-20°C

Time Course of *in vitro* Osteoclast Resorptive Activity Assayed with the OsteoAssay™ Plate and a Telopeptide EIA Kit



The release of collagen peptides from the OsteoAssay™ Plate by differentiating primary human osteoclasts is linear with time. Poietics™ Osteoclast Precursors were seeded onto an OsteoAssay™ Plate at 10,000 cells/well and cultured in medium containing M-CSF +/- soluble RANK ligand. After 5 days of culture, the medium was renewed. Samples of supernatant were harvested after an additional 24, 48 and 96 hours and used in an EIA assay for a telopeptide.

OsteoAssay™ Plate is Superior to Dentine Slices



Comparison of primary human osteoclast function (*in vitro* bone degradation) when cultured on an OsteoAssay™ Plate vs. dentine slices. Poietics™ Human Osteoclast Precursors were seeded at 10,000 cells/well in the presence of either M-CSF alone (undifferentiated control) or both M-CSF and soluble RANK ligand (differentiated) for 5 days. Media were renewed after 5 days and supernatants were harvested after an additional 1 day of culture and assayed for collagen peptides.

Ordering Information – Assays and Reagents

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
PA-1000	PA-1000	OsteoAssay™ Human Bone Plate	-20°C	96-wells

Related Products	Page
Human Osteoclast Precursors	25
OCP Osteoclast Precursor BulletKit™	25
OsteoImage™ Mineralization Assay	275
OsteoLyse™ Assay Kit (Human Collagen)	274

OsteoLyse™ Assay Kit (Human Collagen)

Measure Bone Resorption in Minutes

The OsteoLyse™ Assay Kit provides easy-to-use reagents for quantitatively measuring *in vitro* osteoclast-mediated bone matrix resorption in a high-throughput format. The kit includes a 96-well cell culture plate coated with Europium-labeled human Type I collagen and a bottle of Fluorophore Releasing Agent. Osteoclasts can be seeded onto the OsteoLyse™ Plate using traditional cell culture protocols. The assay directly measures the release of Europium-labeled collagen fragments (resorptive activity) into the osteoclast cell culture supernatant via time resolved fluorescence.

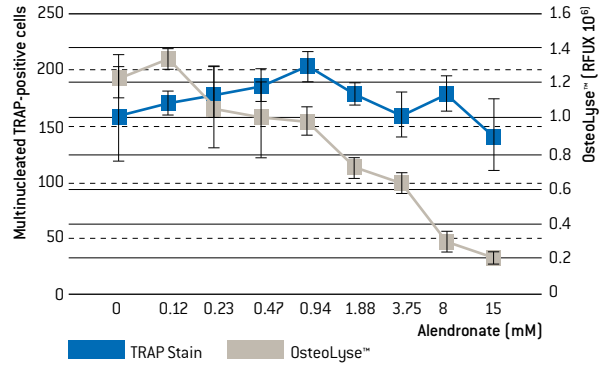
Benefits

- **Convenient** – Human collagen is bound to wells in the plate eliminating the need to purchase bone matrices separately
- **Easy-to-use** – Cells can be seeded onto the surface of the OsteoLyse™ Plate using traditional cell culture protocols
- **Homogeneous** – Resorptive activity is easily measured by simply sampling the cell culture supernatant and counting via time-resolved fluorescence

Applications

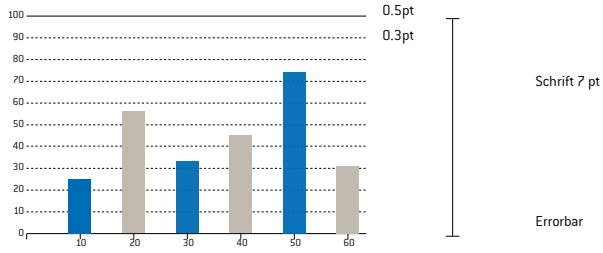
- Osteoporosis
- Bone resorption
- Osteoclast precursor differentiation
- Mature osteoclast enzyme activity
- Cancer research: metastasis/collagen degradation

A comparison of TRAP and OsteoLyse™ Assay Kits in Alendronate-mediated Osteoclast Function



Poietics™ Primary Human Osteoclast Precursors were seeded onto an OsteoLyse™ Plate at 10,000 cells/well and differentiated with M-CSF and soluble RANK ligand in the presence of interferon γ. At day 10 of culture, 10 µl of supernatant was removed and counted. The blue line denotes TRAP data (day 10 multinucleated TRAP-positive cells/well) while the greyline represents OsteoLyse™ Assay data.

Human Osteoclast Activity Measured by CollagenRelease Using the OsteoLyse™ Assay Kit



Poietics™ Primary Human Osteoclast Precursors were seeded onto an OsteoLyse™ Plate at 10,000 cells/well and differentiated with M-CSF and soluble RANK ligand. At days 7, 8, 9 and 10 of culture, 10 µL of supernatant was removed and counted. The blue bars represent counts obtained when the precursors were cultured with M-CSF only.

Ordering Information – Assays and Reagents

Cat. No. NA	Cat. No. EU	Product Name		Storage Conditions	Size
PA-1500	PA-1500	OsteoLyse™ Assay Kit	Human Collagen	4°C to 8°C	96-wells

Related Products	Page
Human Osteoclast Precursors	25
OCP Osteoclast Precursor BulletKit™	25
OsteoImage™ Mineralization Assay	275

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

OsteoImage™ Mineralization Assay

Rapid, Flourescent Assay for Bone Mineralization

The OsteoImage™ Mineralization Assay is a rapid, fluorescent, *in vitro* assay for assessing bone cell mineralization. The assay can quantitate *in vitro* mineralization by osteogenic stem cells, primary osteoblasts, and osteoblast-like cell lines. It is based on specific binding of the fluorescent OsteoImage™ Staining Reagent to the hydroxyapatite portion of bone-like nodules deposited by cells. The assay is sufficiently sensitive to detect the time-dependent increases in mineralization in differentiating osteoblast cultures.

Unlike typical histochemical methods, such as von Kossa and Alizarin Red, neither of which is hydroxyapatite specific, the OsteoImage™ Assay eliminates multiple steps or tedious extraction steps. This latest addition to our line of products for bone research helps you to increase the speed, sensitivity and ease of measuring mineralization in your cell cultures.

Benefits

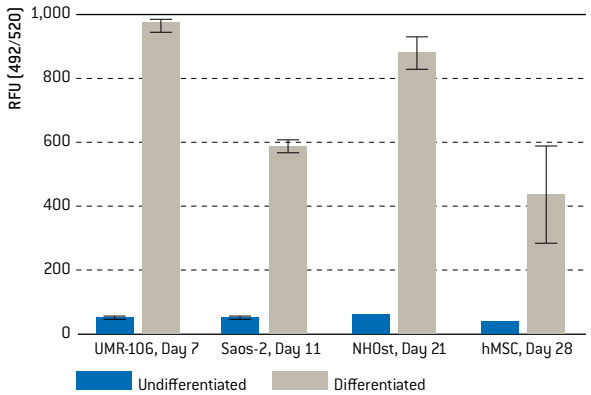
- Delivers qualitative, visual fluorescent microscopy or quantitative plate reader results
- Can be used with primary osteoblasts, osteoblast stem cells, and osteoblast cell lines
- Measures hydroxyapatite, similar to real bone
- Completed in <90 minutes, without tedious extractions
- Sensitive enough to detect time-dependent increases in mineralization in differentiating cells
- Scalable for use in 6-well up to 96-well plates

-20°C

Simple Protocol

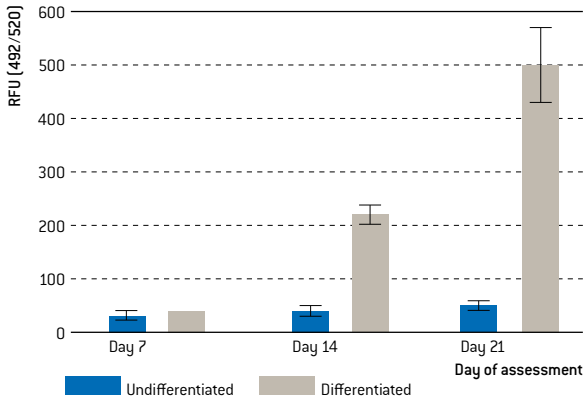
Culture cells
▼
Remove media and wash with PBS
▼
Fix cells (20 minutes and wash)
▼
Add OsteoImage™ Staining Reagent (incubate 30 minutes)
▼
Wash three times
▼
View under fluorescence microscope or read on plate reader

Works with Stem Cells, Primary Cells, and Cell Lines



Osteoblast cell lines, Clonetics™ NHOst – Normal Human Osteoblasts, and osteoblast-differentiated Poietics™ hMSC Human Mesenchymal Stem Cells were evaluated for mineralization with the OsteoImage™ Mineralization Assay on a 96-well plate reader.

Detects Mineralization with Time



NHOst – Normal Human Osteoblasts were seeded at 3,200 cells/well in a 96-well plate. Cells were cultured as undifferentiated control cells or with differentiation factors. Mineralization was quantitated on a plate reader after staining with the OsteoImage™ Assay on days 7, 14 and 21.

Ordering Information – Assays and Reagents

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
PA-1503	PA-1503	OsteoImage™ Mineralization Assay	-20°C	5 × 96-wells

Related Products	Page
Human Mesenchymal Stem Cells	29
Human Osteoblast Cells and Growth Medium	84

BioAssay Accessory Products

Clear Bottom, White-Walled Tissue Culture Plates

Clear Bottom, White-walled Tissue Culture Plates are white-walled 96-well plastic plates designed specifically for use with any bioluminescent bioassay kit.

The ATP Standard

The ATP Standard is a specialized aqueous preparation of adenosine triphosphate (ATP) and is primarily intended for use in research to calibrate ATP assays based on the luciferase bioluminescence technique. Each vial contains 5 ml of 10 µM ATP.

-20°C

Ordering Information – Labware

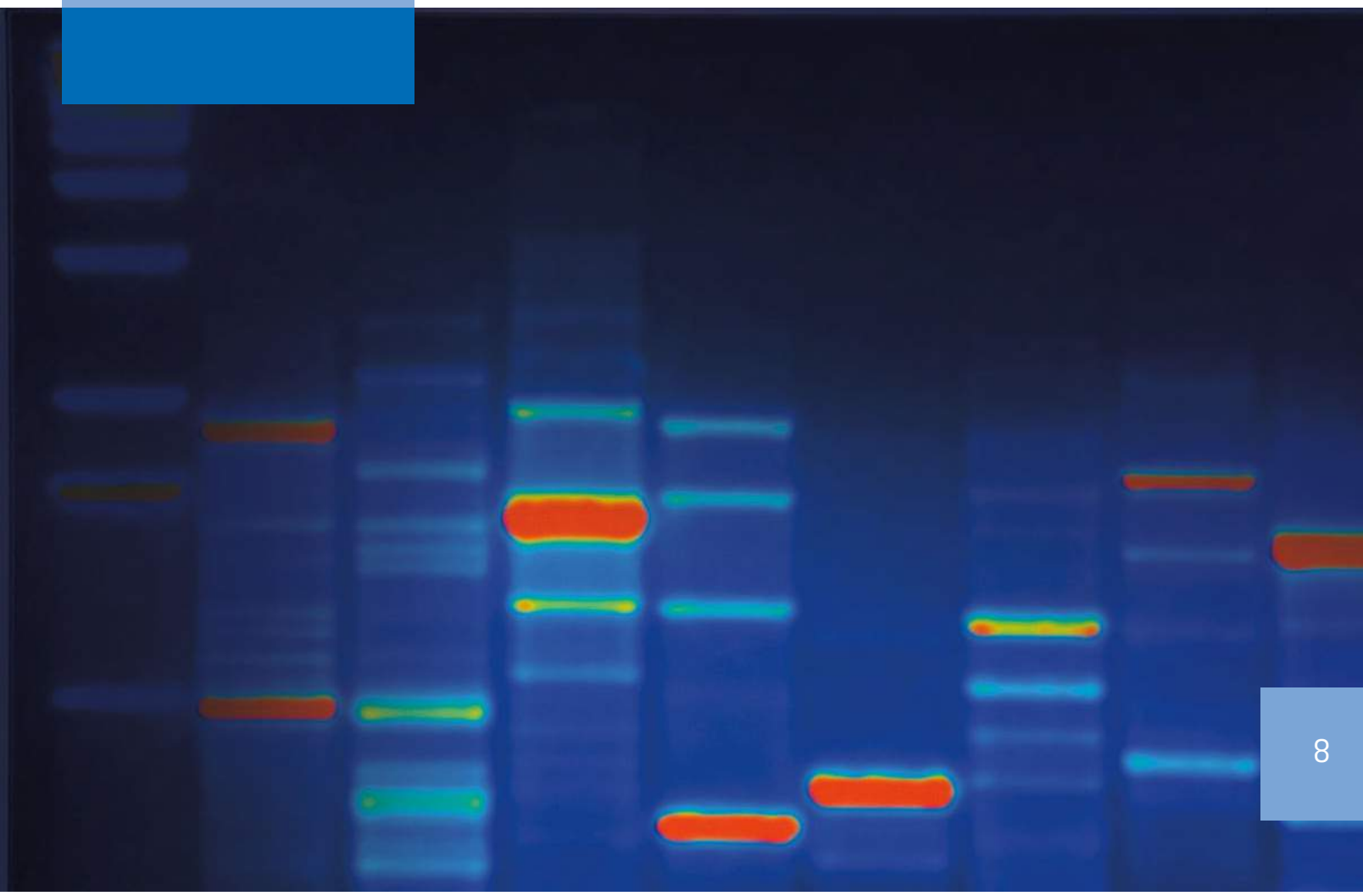
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
LT27-102	LT27-102	Tissue Culture Plates	Clear bottom, white walled	25 plates

Ordering Information – Kits

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
LT27-008	LT27-008	ATP Standard	-20°C	5 mL

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

8 Electrophoresis and Analysis



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Electrophoresis and Analysis

Nucleic Acid Electrophoresis

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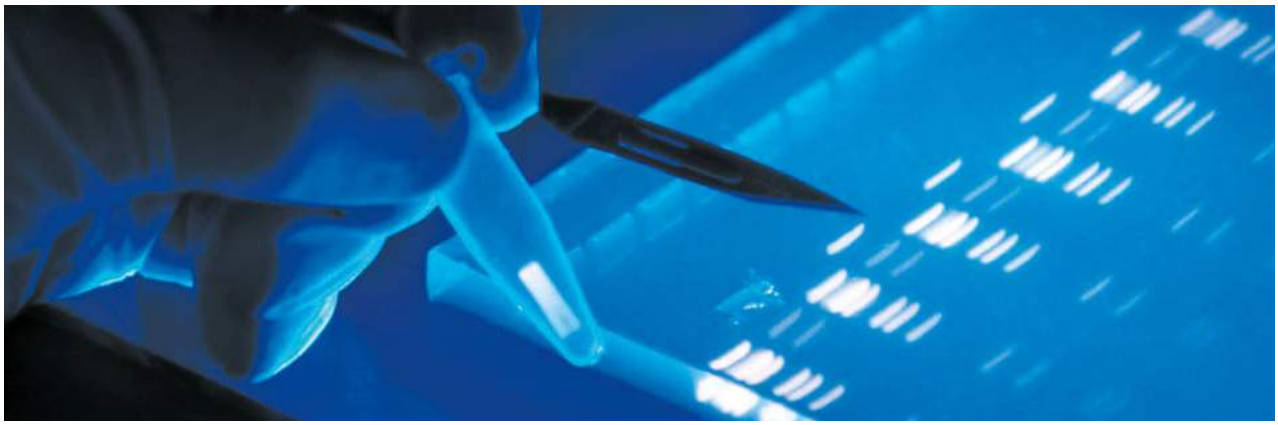
8

Nucleic Acid Electrophoresis

From the Very Beginning to the Next Innovation



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Introduction

Lonza is the leading innovator and world's most trusted supplier of agarose and precast gels. We are experts in protein and nucleic acid electrophoresis, bringing a strong history of innovation and reliability to your most important research. Our well known product brands set the standard in quality, purity and performance for electrophoresis.

- SeaKem®, NuSieve™ and MetaPhor™ Agarose
- FlashGel™ System
- Reliant™, Latitude™ and PAGEr™ Precast Gels
- AccuGENE™ Buffers
- GelBond® Gel Support Film

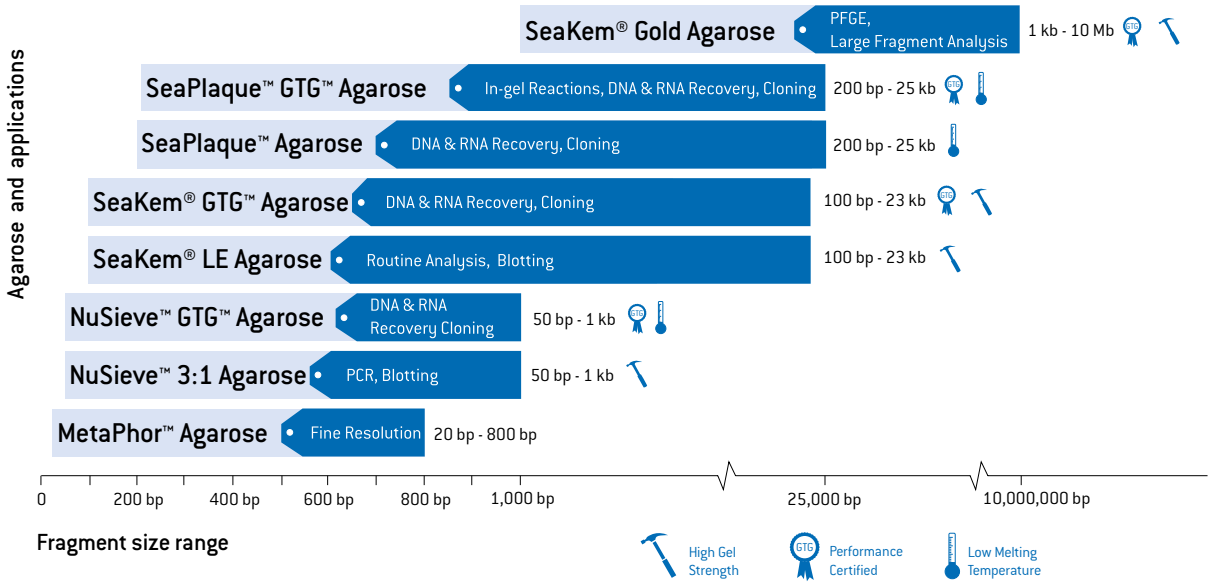
Covering the most extensive range of applications, our products are optimized for the unique requirements of your most critical molecular biology techniques. When our standard products do not completely fit your needs, inquire about our custom capabilities to find a product that does.

Agarose Selection Guide

Selecting the best agarose for your application can minimize opportunity for error, optimize results, and even reduce cost. We offer a wide range of agarose types that are specifically engineered to optimize results by fragment size, sample type and application. The selection tools below will get you started. The following pages will guide you to the right concentration, buffer and marker to use for best performance in your experiment. If you require additional support, visit our online Sourcebook for Electrophoresis.

www.lonza.com/sourcebook

Choose the Agarose that is Right for You



Agarose Selection Guide

Continued

Agarose and Compatible Techniques

Recovery method	SeaKem® LE	SeaKem® GTG™	SeaPlaque™	SeaPlaque™ GTG™	NuSieve™ 3:1	NuSieve™ GTG™	MetaPhor™	SeaKem® Gold	SeaPrep™	InCert™	I.D.NA™
In-gel reactions				■		■					
β-Agarase			■	■		■					
Phenol/chloroform			■	■		■					
Recovery columns	■	■	■	■	■	■	■	■			■
Electroelution	■	■	■	■	■	■	■	■			■
Freeze/squeeze	■	■	■	■	■	■	■	■			■
Blotting											
Southern <1 kb					■						
Southern >1 kb	■										
Northern <1 kb					■						
Northern >1 kb	■										
Specialty applications											
Viral plaque assays			■	■							
Preparation of megabase samples										■	
PFGE								■		■	
Cell culture			■	■					■		
Encapsulation and embedding of cells									■		
DNA identity testing											■
Comet assays	■	■									

Our agarose is GUARANTEED DNase/RNase-free

SeaKem® LE Agarose

The Standard for Routine Analysis

High Gel Strength

SeaKem® LE Agarose is the ideal multipurpose, molecular biology grade agarose for any DNA or RNA application.

Benefits

- Wide resolution range – 100 bp–23 kb
- High gel strength – ideal for blotting
- Consistent lot-to-lot performance

Applications

- Broad range fragment separation
- Southern and Northern blotting
- PCR greater than 1 kb
- Immunoprecipitation techniques
- Baculovirus screening and colony lifts

18°C to 26°C

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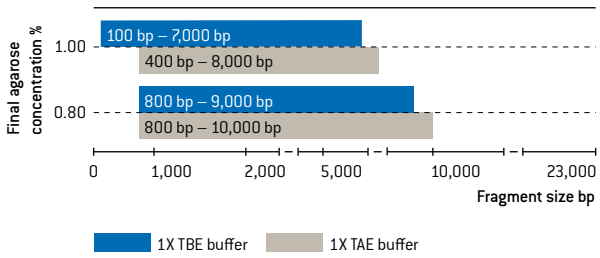
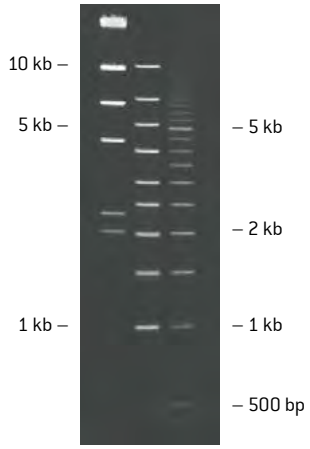
SeaKem® LE Agarose



1% SeaKem® LE Agarose Gel in TAE Buffer



Lane 1: *Hind* III digest of lambda DNA
Lane 2: DNA marker 1 to 10 kb (Lonza)
Lane 3: 500 bp DNA ladder (Lonza)

Ordering Information – SeaKem® LE Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50001	50001	SeaKem® LE Agarose	18°C to 26°C	25 g
50002	50002	SeaKem® LE Agarose	18°C to 26°C	100 g
50000	50000	SeaKem® LE Agarose	18°C to 26°C	125 g
50004	50004	SeaKem® LE Agarose	18°C to 26°C	500 g
50005	50005	SeaKem® LE Agarose	18°C to 26°C	1 kg

Related Products	Page
AccuGENE™ 1 M Tris HCl Buffer	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

MetaPhor™ Agarose

The Highest Resolution Agarose Available

MetaPhor™ Agarose offers twice the resolution capability of standard agarose for PCR, STR and AmpFLP analysis. This intermediate melting temperature agarose rivals polyacrylamide and is capable of resolving DNA fragments differing in size by 2% between 20 bp and 800 bp.

Benefits

- Fine separation of fragments 20 bp–800 bp
- Rivals the resolution capability of polyacrylamide
- Eliminates hazards associated with polyacrylamide

Applications

- Small PCR analysis
- STR analysis
- RT-PCR

Performance and Quality Tests

- DNA resolution: 4 bp resolution of DNA fragments at 200 bp and 16 bp resolution at 800 bp in TBE buffer
- Gel background: gel exhibits low background fluorescence after ethidium bromide staining
- DNA binding: none detected

18°C to 26°C

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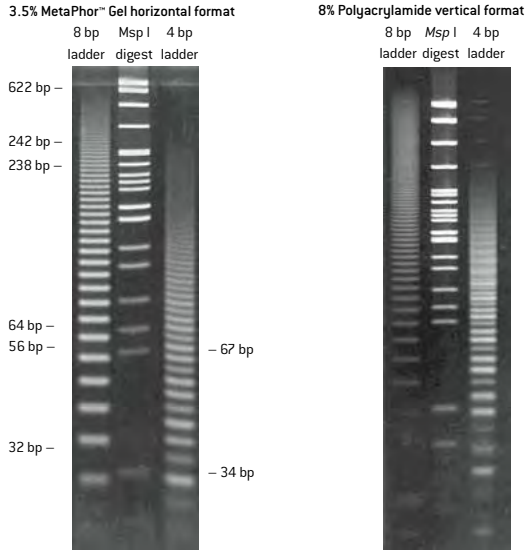
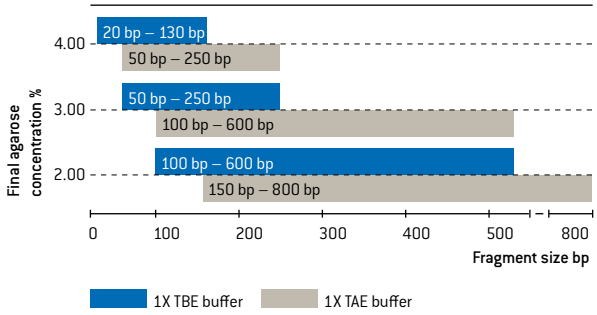
MetaPhor™ Agarose



Resolution of DNA Ladders in MetaPhor™ Agarose



DNA ladders with 4 bp or 8 bp step sizes were prepared by ligation of Bgl II linkers. Aliquots of 0.8 µg of the ladders were separated on a 3.5% MetaPhor™ Agarose gel in a horizontal format and compared to an 8% polyacrylamide gel run in a vertical format in TBE buffer. The horizontal gel (15 cm × 20 cm and 3.0 mm thick) was run at 6.7 V/cm for 4 hours at 15°C. The vertical gel (10 cm × 20 cm and 1.0 mm thick) was run at 8 V/cm for 2 hours.

Ordering Information – MetaPhor™ Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50181	50181	MetaPhor™ Agarose	18°C to 26°C	25 g
50180	50180	MetaPhor™ Agarose	18°C to 26°C	125 g
50184	50184	MetaPhor™ Agarose	18°C to 26°C	500 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ 1 M Tris HCl Buffer	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

NuSieve™ 3:1 Agarose

The Reliable Choice for PCR Analysis

High Gel Strength

NuSieve™ 3:1 Agarose was the first and still is the most reliable choice for separating and resolving PCR and RT-PCR fragments. This molecular biology grade agarose produces strong, easy-to-handle gels, making it ideal for blotting of small fragments.

Benefits

- Exceptional resolution of small fragments between 50 bp and 1 kb
- Superior gel strength for blotting
- Widely cited as the choice for PCR analysis

Applications

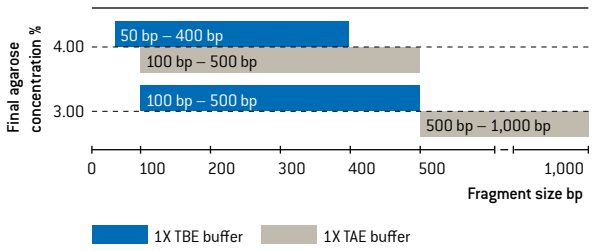
- Small DNA and RNA fragment analysis
- Blotting of small fragments
- RT-PCR and Genotyping

Performance and Quality Tests

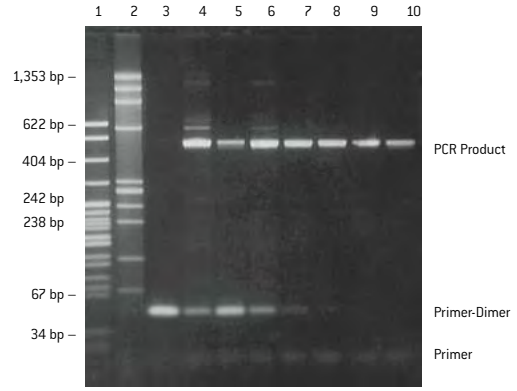
- Resolution: DNA fragments ≤1,000 bp are finely resolved after electrophoresis
- Gel background: gel exhibits low background fluorescence after ethidium bromide staining
- DNA binding: none detected

18°C to 26°C

NuSieve™ 3:1 Agarose



PCR Products on a NuSieve™ 3:1 Agarose Gel



A 550 bp sequence from lambda DNA was amplified (25 cycles) using primers and Taq DNA polymerase supplied in the GeneAmp® Kit (Roche Molecular Systems). PCR products and controls were electrophoresed on a 4% NuSieve™ 3:1 Agarose gel in TAE buffer at 5 V/cm for 3 hours. Lane 1, Msp I digest of pBR322 DNA (1.5 µg); lane 2, Hae III digest of øX174 DNA (1.5 µg); lane 3, no DNA control; lanes 4–9, PCR products resulting from different reaction conditions (7 µL of 100 µL reaction mixture); and lane 10, a positive control where kit template was added.

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www.lonza.com/sourcebook

Ordering Information – NuSieve™ 3:1 Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50091	50091	NuSieve™ 3:1 Agarose	18°C to 26°C	25 g
50090	50090	NuSieve™ 3:1 Agarose	18°C to 26°C	125 g
50094	50094	NuSieve™ 3:1 Agarose	18°C to 26°C	500 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ 1 M Tris HCl Buffer	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

NuSieve™ GTG™ Agarose

Performance Certified for Small Fragment Recovery and In-gel Reactions

Low Melting Temperature

Performance Certified

NuSieve™ GTG™ Agarose provides optimal separation and resolution of PCR and RT-PCR fragments. This low melting (≤65°C) temperature agarose is easy-to-handle and can be used for cloning procedures directly from remelted agarose. Genetic Technology Grade™ Agarose is quality tested to certify performance.

Benefits

- Fine resolution of small fragments between 50 bp and 1 kb
- Performance certified for digestion and ligation

Applications

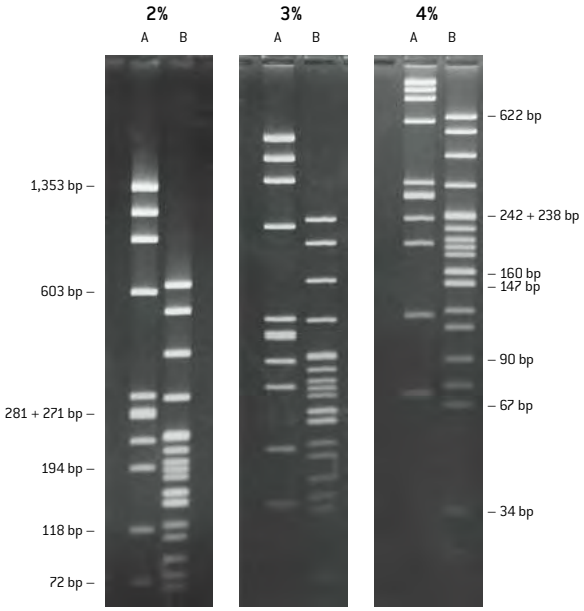
- Analysis and recovery of small DNA fragments
- In-gel PCR and In-gel ligations/transformations

Performance and Quality Tests

- Enzymatic activity in the presence of remelted gel: T4 DNA ligase and transformation test
- Resolution: DNA fragments ≤1,000 bp are finely resolved after electrophoresis
- Gel background: gel exhibits low background fluorescence after ethidium bromide staining
- DNase and RNase activity: none detected
- DNA binding: none detected

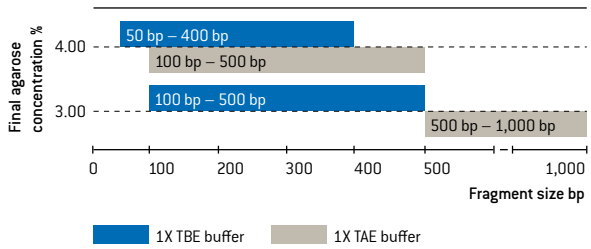
18°C to 26°C

Fine Resolution of Low Molecular Weight DNA Fragments in NuSieve™ GTG™ Agarose



DNA fragments were separated in 2%, 3%, and 4% NuSieve™ GTG™ Agarose gels in 1X TBE buffer. Lane A: Hae III digest of øX174 DNA, 0.5 µg/lane. Lane B: Msp I digest of pBR322 DNA, 0.5 µg/lane. Running conditions: 1X TBE at 5 V/cm.

NuSieve™ GTG™ Agarose



Ordering Information – NuSieve™ GTG™ Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50081	50081	NuSieve™ GTG™ Agarose	18°C to 26°C	25 g
50080	50080	NuSieve™ GTG™ Agarose	18°C to 26°C	125 g
50084	50084	NuSieve™ GTG™ Agarose	18°C to 26°C	500 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ 1 M Tris HCl Buffer	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

SeaPlaque™ GTG™ Agarose

Performance Certified for Large Fragment Recovery and In-gel Reactions

Low Melting Temperature

Performance Certified

Confidently resolve fragments from 200 bp to 25 kb prior to PCR, cloning, digesting, or sequencing in the presence of re-melted SeaPlaque™ GTG™ Agarose, without additional purification steps. This low-melting temperature (≤65°C) Genetic Technology Grade™ Agarose is quality tested to certify performance.

Benefits

- Optimal separation range for DNA and RNA recovery of fragments: 200 bp to 25 kb
- Performance certified

Applications

- Analysis and recovery of large DNA fragments
- In-gel PCR and In-gel ligations and transformations
- DNA and RNA digestion

Performance and Quality Tests

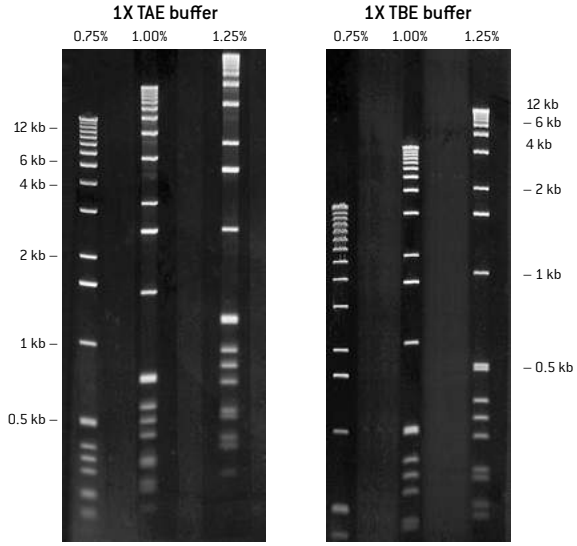
- Enzymatic activity in the presence of remelted gel:
  - T4 DNA ligase and transformation test
  - *Hin*d III and *Eco*R I restriction digestion test
- Fine resolution of DNA fragments ≥1,000 bp with low background after ethidium bromide staining
- DNase and RNase activity: none detected
- DNA binding: none detected

18°C to 26°C

Pages 455–458

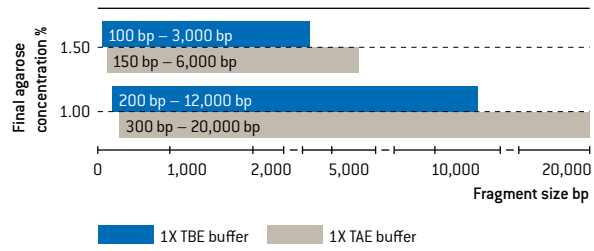
www.lonza.com/sourcebook

Resolution Performance of SeaPlaque™ GTG™ Agarose



Separation of DNA markers in 0.75% to 1.25% SeaPlaque™ GTG™ Agarose gels in 1X TAE and TBE buffers. 1 kb DNA ladder, 1 µg/lane, DNA unheated prior to loading. The gels were cast in a 25.5 cm framing gel of 1% SeaKem® GTG™ Agarose in a submarine chamber and run under 5 mm of buffer overlay at 5 V/cm for 3 hours, 40 minutes (TBE buffer) and 4 hours, 30 minutes (TAE buffer).

SeaPlaque™ GTG™ Agarose



Ordering Information – SeaPlaque™ Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50111	50111	SeaPlaque™ GTG™ Agarose	18°C to 26°C	25 g
50110	50110	SeaPlaque™ GTG™ Agarose	18°C to 26°C	125 g
58001	58001	ß-Agarase	18°C to 26°C	100 units
58005	58005	ß-Agarase	18°C to 26°C	500 units

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ 1 M Tris HCl Buffer	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

SeaKem® GTG™ Agarose

Performance Certified for Large Fragment Recovery

High Gel Strength

Performance Certified

SeaKem® GTG™ Agarose ensures reliable digestion and ligation from recovered DNA or RNA fragments from 100 bp to 23 kb. Our Genetic Technology Grade™ Agarose is quality tested to certify performance.

Applications

- Best choice for DNA and RNA recovery and cloning of fragments 100 bp to 23 kb

Performance and Quality Tests

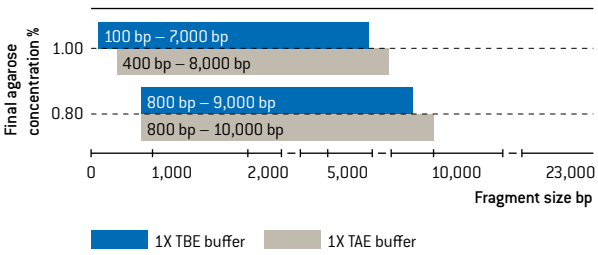
- Restriction endonuclease digestion test: *Eco*R I and *Hin*d III are tested for complete digestion of electroeluted, linearized pBR322 DNA
- Ligation of recovered DNA
- Fine resolution of DNA fragments ≥1,000 bp with low background after ethidium bromide staining
- DNase and RNase activity: none detected
- DNA binding: none detected

18°C to 26°C

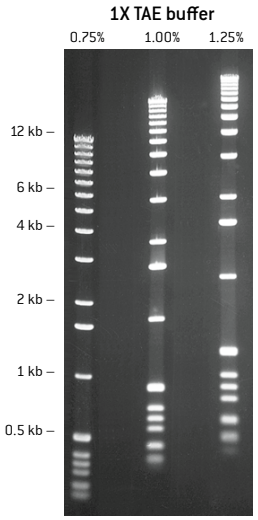
Pages 455–458

www.lonza.com/sourcebook

SeaKem® GTG™ Agarose

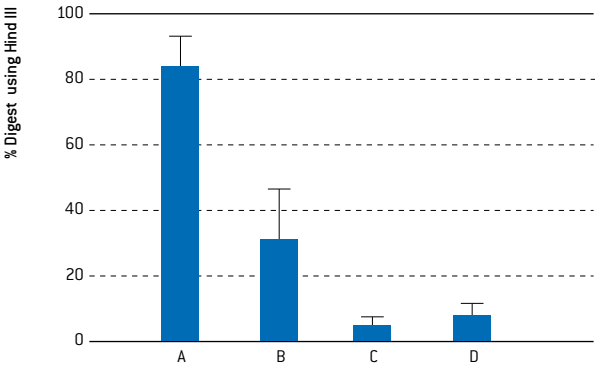


Resolution Performance of SeaKem® GTG™ Agarose



1 kb DNA ladder (Lonza) 1 µg/lane, unheated

Efficient Digestions after Recovery



(A) SeaKem® GTG™ Agarose, (B) Competitor's agarose, (C) Competitor's agarose, (D) Competitor's agarose

Ordering Information – SeaKem® GTG™ Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50071	50071	SeaKem® GTG™ Agarose	18°C to 26°C	25 g
50070	50070	SeaKem® GTG™ Agarose	18°C to 26°C	125 g
50074	50074	SeaKem® GTG™ Agarose	18°C to 26°C	500 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ 1 M Tris HCl Buffer	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

SeaPlaque™ Agarose

The Original Low-melting Temperature Agarose

Low Melting Temperature

SeaPlaque™ Agarose is the original low-melting temperature agarose and has been a staple in molecular biology labs for over 40 years. This molecular biology grade agarose produces gels with greater sieving capabilities from 200 bp to 25 kb, and with higher clarity than standard melting temperature agarose. Ideal for preparative DNA and RNA electrophoresis.

Benefits

- Ideally suited for DNA and RNA recovery
- Also ideal for cloning of tissue culture cells and viral plaque assays

Applications

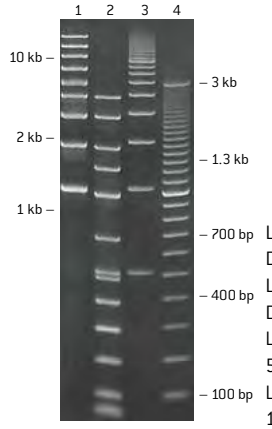
- Preparative DNA and RNA electrophoresis
- Viral plaque assays
- Cell culture
- Separating proteins >600 kDa

18°C to 26°C

Pages 455–458

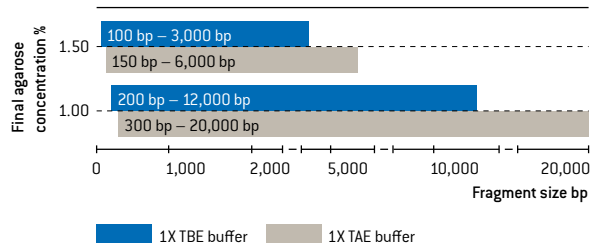
www.lonza.com/sourcebook

1% SeaPlaque™ Agarose Gel



Lane 1:
DNA marker 1 to 10 kb (Lonza)
Lane 2:
DNA marker 50 to 2,500 bp (Lonza)
Lane 3:
500 bp DNA ladder (Lonza)
Lane 4:
100 bp Extended Range DNA ladder (Lonza)

SeaPlaque™ Agarose



Ordering Information – SeaPlaque™ Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50101	50101	SeaPlaque™ Agarose	18°C to 26°C	25 g
50100	50100	SeaPlaque™ Agarose	18°C to 26°C	125 g
58001	58001	ß-Agarase	18°C to 26°C	100 units
58005	58005	ß-Agarase	18°C to 26°C	500 units

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ 1 M Tris HCl Buffer	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

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ß-Agarase

Recovers DNA and RNA from Low-melting Temperature Agarose

ß-Agarase is an enzyme that will completely digest the polysaccharide backbone of molten agarose into alcohol soluble oligosaccharides. DNA electrophoresed in low melting temperature agarose gels can be recovered after the gel is melted and then digested with this enzyme. Any remaining agarose oligosaccharides will not gel or interfere with subsequent DNA manipulations such as cloning, labeling, restriction digestion, or sequencing.

Concentration

ß-Agarase is supplied at 1,000 units/mL in a buffer consisting of 50% glycerol, 50 mM Tris/HCl, 100 mM NaCl, and 0.1% Triton® X-100 at pH 7.5.

Unit Definition

One unit contains the amount of enzyme necessary to completely digest 200 mg of molten 1% SeaPlaque™ GTG™ Agarose gel prepared in 40 mM Bis Tris/HCl, 40 mM NaCl, 1 mM EDTA (pH 6.0) at 40°C in 1 hour.

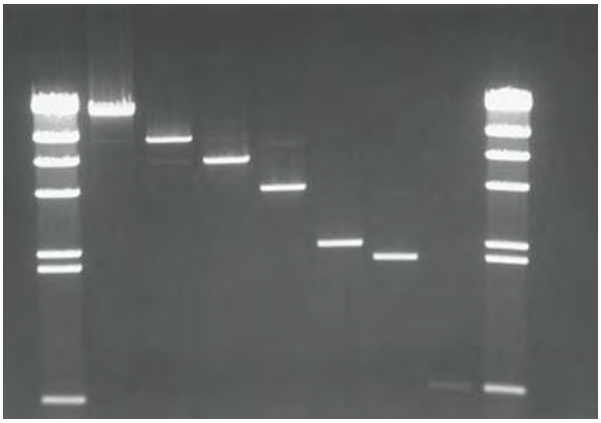
Performance and Quality Tests

– No detectable DNase or RNase

18°C to 26°C

www.lonza.com/sourcebook

Recovery of DNA from 1% SeaPlaque™ GTG™ Agarose with ß-Agarase



***Hin*d III-digested lambda DNA gel stained with ethidium bromide and the DNA bands excised.** Gel digested with ß-Agarase, and DNA recovered by ethanol precipitation. The recovered DNA was applied to a 1% SeaKem® GTG™ Agarose gel in 1X TBE buffer and separated by electrophoresis. A photograph of the second gel stained with ethidium bromide is shown.

Ordering Information – ß-Agarase

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
58001	58001	ß-Agarase	18°C to 26°C	100 units
58005	58005	ß-Agarase	18°C to 26°C	500 units

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
NuSieve™ GTG™ Agarose	285
SeaPlaque™ Agarose	288
SeaPlaque™ GTG™ Agarose	286

SeaKem® Gold Agarose

Performance Certified for Rapid Resolution of Megabase DNA by PFGE

High Gel Strength

GTG Performance Certified

SeaKem® Gold Agarose is ideal for separating very large DNA fragments or doing pulsed field gel electrophoresis (PFGE). This Genetic Technology Grade™ Agarose is ideal for rapid resolution of megabase DNA, decreasing run times by up to 50% for PFGE.

Benefits

- Capable of rapid separation of large DNA from 30 kb to 50 kb by horizontal electrophoresis or 50 kb to 10 Mb by PFGE
- Good multipurpose, high gel strength agarose for separations ≥1,000 bp
- Specially manufactured to create a strong gel that is easy-to-handle
- Guaranteed DNase and RNase-free

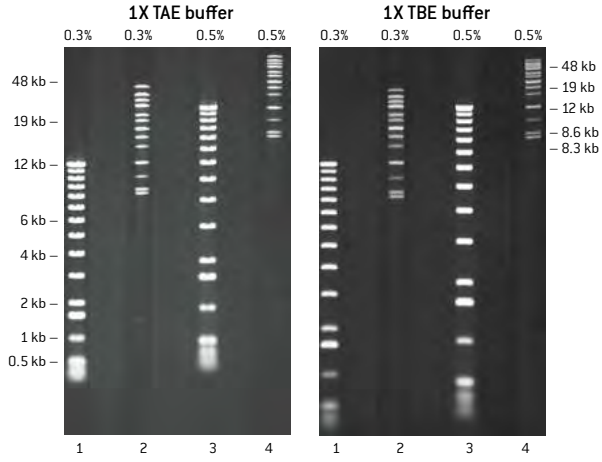
Applications

- Large fragment separation
- Pulsed field gel electrophoresis
- Blotting of megabase DNA
- Effective for separating proteins ≥600 kDa

Performance and Quality Tests

- Relative DNA mobility: 1.3 under PFGE conditions (SeaKem® LE Agarose = 1.0)
- Restriction endonuclease digestion test: *Eco*R I and *Hind* III tested for complete digestion of recovered DNA
- Ligation of recovered DNA
- Resolution: DNA fragments ≥1,000 bp are finely resolved after electrophoresis
- Gel background: gel exhibits low background fluorescence after ethidium bromide staining
- DNase and RNase activity: none detected
- DNA binding: none detected

Performance of SeaKem® Gold Agarose for DNA ≤ 50 kb



DNA markers separated in 0.3% and 0.5% SeaKem® Gold Agarose gels in 1X TAE and TBE buffers. Lanes 1 and 3 are 1 kb ladders, 1 µg/lane, DNA unheated prior to loading. Lanes 2 and 4 are high molecular weight markers (8.3, 8.6, 10.1, 12.2, 15.0, 17.0, 19.4, 22.6, 24.8, 29.9, 33.5, 38.4, 48.5 kb), 0.3 µg/lane, DNA heated 10 minutes at 65°C prior to loading. Gels were cast in a 25.5 cm framing gel of 1% SeaKem® GTG™ Agarose in a submarine chamber and run under 5 mm of buffer overlay at 1 V/cm for 16 hours (TAE buffer), and 20 hours (TBE buffer).

18 to 26°C

Pages 455–460

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Ordering Information – SeaKem® Gold Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50152	50152	SeaKem® Gold Agarose	18°C to 26°C	25 g
50150	50150	SeaKem® Gold Agarose	18°C to 26°C	125 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
InCert™ Agarose and Megabase DNA Standards	291
AccuGENE™ Buffers	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314

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InCert™ Agarose and Megabase DNA Standards

Used to Prepare Chromosomal DNA for PFGE

InCert™ Agarose is a low-gelling temperature agarose, certified for use in the preparation and digestion of chromosomal DNA prior to pulsed field gel electrophoresis (PFGE).

Our **Megabase DNA Standards** are specially prepared and tested chromosomal DNA standards offered in InCert™ Agarose gel plug format for easy handling during PFGE.

Benefits

- Certified performance for chromosomal DNA preparation and restriction endonuclease digestion
- Performance tested for reliable PFGE
- Saves time – standards are ready-to-use

Applications

- Pulsed field gel electrophoresis

Performance and Quality Tests for InCert™ Agarose

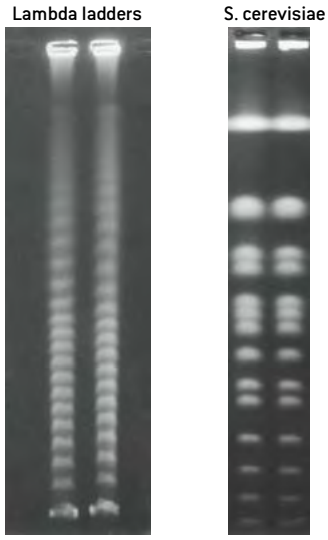
- Restriction endonuclease digestion in agarose gel plugs test: enzymes tested on *E. coli* DNA: *Eco*R I, and *Hin*d III
- DNase activity: none detected

InCert™ Agarose: 18°C to 26°C
Megabase DNA Standards: 2°C to 8°C

Pages 455–460

www.lonza.com/sourcebook

Performance of Megabase DNA Standards in PFGE



Lambda DNA ladders and *S. cerevisiae* and DNA Standards were run on the Bio-Rad® CHEF-DR® III System.
Running conditions:
Lambda ladders: 1% SeaKem® GTG™ Agarose, 0.5X TBE, switch angle 120°, 6 V/cm, ramped switch time from 50–90 seconds over 22 hours.
S. cerevisiae: 1% SeaKem® GTG™ Agarose, 0.5X TBE, switch angle 120°, 6 V/cm, ramped switch time from 40–100 seconds over 24 hours.

Ordering Information – InCert™ Agarose and Megabase Standards

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
50121	50121	InCert™ Agarose		18°C to 26°C	1 g
50123	50123	InCert™ Agarose		18°C to 26°C	5 g
50401	50401	Lambda DNA Ladder	50 kb to 873 kb		5 plugs (10 ± 2 µg DNA/plug)
50411	50411	*Saccharomyces cerevisiae* DNA Standard	220 kb to approximately 1 Mb chromosomal DNA	2°C to 8°C	5 plugs (10 ± 2 µg DNA/plug)

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ Buffers	316

SeaKem® ME Agarose

Ideal for Serum Protein and IEP Analysis

SeaKem® ME Agarose is the ideal choice for serum protein electrophoresis and immunoelectrophoresis, and may be used for DNA electrophoresis.

18°C to 26°C

Pages 455–460

www.lonza.com/sourcebook

Benefits

- Enhanced resolution in serum protein electrophoresis
- High gel clarity and minimal non-specific binding

Applications

- Serum protein electrophoresis
- Immunoelectrophoresis
- Nucleic acid electrophoresis

Ordering Information – SeaKem® ME Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50011	50011	SeaKem® ME Agarose	18°C to 26°C	25 g
50010	50010	SeaKem® ME Agarose	18°C to 26°C	125 g
50014	50014	SeaKem® ME Agarose	18°C to 26°C	500 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

SeaPrep™ Agarose

Ideal for Cell Culture Applications

SeaPrep™ Agarose is a unique ultra-soft agarose, ideal for high efficiency hybridoma cloning. It is also used for expanding cDNA libraries in a strictly representative fashion, decreasing the possibility that less abundant clones vanish during amplification due to differential rates of replication.

Applications

- Cell culture
- Hybridoma cloning
- Encapsulation/embedding of cells

18°C to 26°C

Pages 455–460

www.lonza.com/sourcebook

Specifications

- Melting temp: ≤50°C at 1%
- Gelling temp: 8°C to 17°C at 0.8%
- Gel Strength: >75 g/cm^2 at 2%

Ordering Information – SeaPrep™ Agarose

Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50302	50302	SeaPrep™ Agarose	18°C to 26°C	25 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ Buffers	316

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I.D.NA™ Agarose

Designed for Identity Testing

I.D.NA™ Agarose is specially manufactured for DNA identity testing. For reliable separation of VNTRs, HVRs, RFLPs, and DNA size standards, it is a perfect match for your DNA typing tests.

Benefits

- Performance certified to assure lot-to-lot reliability for DNA identity testing
- Crisp DNA separation to accurately discriminate DNA fragments
- Strong, easy-to-handle gels allow for trouble-free high efficiency blotting

Applications

- DNA identity testing

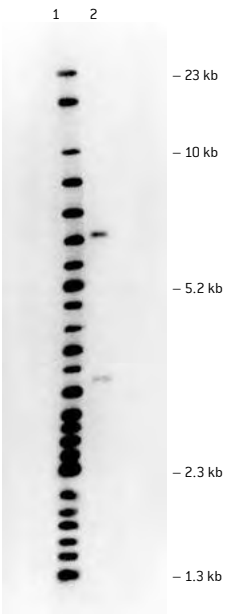
Performance and Quality Tests

- DNase and RNase activity: none detected
- DNA binding: none detected

18°C to 26°C

Page 456 (Analytical specifications)

Resolution and Transfer Performance of I.D.NA™ Agarose



An autoradiogram of DNA size standards (LIFECODES Corp.) and *Hae* III-digested K562 DNA probed with D4S139 (Invitrogen). DNA was electrophoresed at 1 V/cm for 16 hours in a 1% I.D.NA™ Agarose gel, transferred, and probed. Lane 1: DNA size standards; Lane 2: alleles detected with D4S139.

Ordering Information – I.D.NA™ Agarose

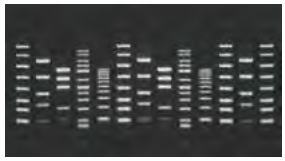
Cat. No. NA	Cat. No. EU	Product Name	Storage Conditions	Size
50170	50170	I.D.NA™ Agarose	18°C to 26°C	125 g

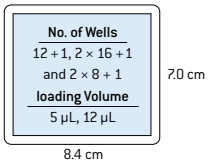
Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ Buffers	316
DNA Ladders and Markers	313
GelStar® and SYBR® Green Nucleic Acid Gel Stains	310,314
MetaPhor™ Agarose	283

We offer a complete family of precast agarose gels for DNA and RNA electrophoresis. Our unique gel options cover the full range of separation needs, from ultra-fast PCR analysis and recovery, to fine resolution and high-throughput separations. Our custom manufacturing capabilities can support the requirements of nearly any application. All Lonza Gels are precision manufactured with our high quality SeaKem® and NuSieve™ Agarose and functionally tested for consistent performance.

FlashGel™ System

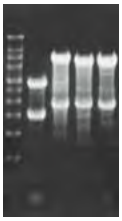


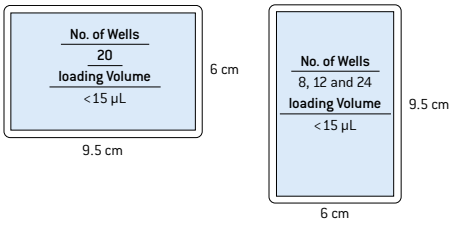


- **Five Minute DNA Separation:**
- Separate DNA 10 bp to 10 kb and RNA 0.5 kb to 9 kb
- Watch DNA migrate in real time without UV light
- Recover samples directly, without purification
- Run 15–34 samples

See page 295 for a complete product description.

Reliant™ Minigels

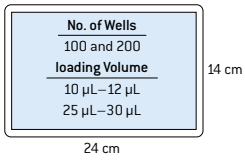




- **Small Format Gels for DNA and RNA:**
- Run 8–24 samples
- Ideal for blotting and recovery
- Fits standard horizontal chambers

See page 302 for a complete product description.

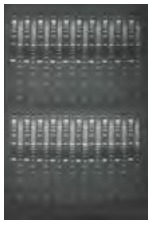
Latitude™ HT Gels

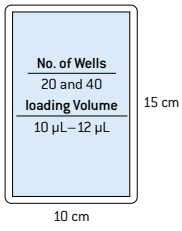


- **Large Format Gels for DNA:**
- Run 100–200 samples
- Ideal for high-throughput screening of DNA samples
- Fits standard horizontal chambers

See page 304 for a complete product description.

Latitude™ Midigels



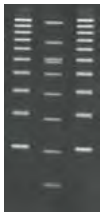


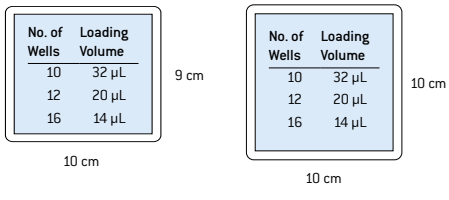
- **Medium Format Gels for DNA and RNA:**
- Run 20–40 samples
- Ideal for routine analysis, blotting and recovery
- Fits standard horizontal chambers

See page 306 for a complete product description.

Contact Scientific Support to inquire about custom precast gels.

PAGEr™ Gold TBE Gels





- **Vertical Polyacrylamide Gels for DNA:**
- Run 10–16 samples
- Ideal for fine resolution
- Easy to load and open
- Fits most standard mini-vertical chambers

See page 307 for a complete product description.

FlashGel™ System

Fast, Sensitive, Simple Analysis, Recovery, and Documentation of DNA and RNA

The FlashGel™ System gets straight to your results. Simply load samples, watch bands migrate and get data in as little as 2 minutes. Say goodbye to gel preparation, band excision, purification, and UV light. Complete separation, recovery and documentation safely, at the bench, in minutes.

- **5 Minute Separation and Recovery**
  - See bands in as little as 2 minutes
  - Recover samples directly, without UV light, band excision or purification
- **Real**-time Separation and Documentation
  - Watch band migration as it happens
  - Photograph gels at the bench, without DNA damaging UV light
- **Outstanding Sensitivity and Resolution**
  - 5–20 times more sensitive than ethidium bromide; detect <0.1 ng DNA or <10 ng total RNA
  - Clean, sharp separation and straight, uniform sample lanes

FlashGel™ Dock and Cassettes

FlashGel™ System

The FlashGel™ System consists of enclosed, disposable, precast agarose gel cassettes and a combination electrophoresis and transilluminator unit.

- **FlashGel™ Cassettes contain precast, prestained agarose gels and buffer** – no need for gel preparation, buffer addition or gel staining
- The FlashGel™ Dock is an electrophoresis apparatus with a built-in visible light transilluminator that provides both separation and detection
- The FlashGel™ Camera is a compact camera system designed to photograph FlashGel™ Cassettes right at the bench
- FlashGel™ Markers are recommended for best performance

www.flashgel.com

FlashGel™ System for DNA

5 Minute DNA Analysis

The FlashGel™ System for DNA is the ideal sample screening tool. Check up to 34 PCR or restriction fragments quickly, without having to plan your day around agarose gels.

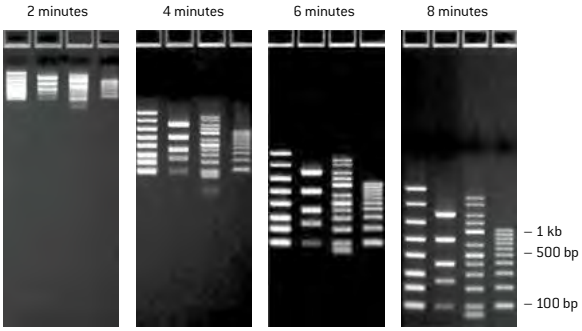
Fast, Simple Procedure

1. Insert cassette into dock.
2. Pre-load wells with distilled or deionized water.
3. Load samples.
4. Plug in and turn on light and electrophoresis voltage.
5. Watch until desired separation is achieved.
6. Photograph.

5 Minute Separation

- The FlashGel™ System provides high voltage separation of fragments (275 V for 2–7 minutes, depending upon fragment size)

Separation at Various Run Times on the FlashGel™ System



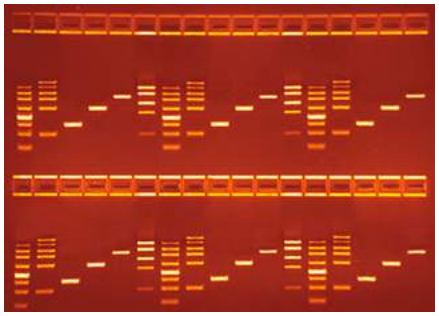
Markers run on a 1.2% FlashGel™ Cassette, 12+1-well format, 275 V for times as shown. Sample lanes from left to right: FlashGel™ DNA Marker (100 bp–4 Kb), FlashGel™ QuantLadder, Lonza 50–2500 bp Marker, Lonza 100 bp Ladder.

FlashGel™ System for DNA

Continued

Real-time Visualization

– Built-in illumination, allows you to view DNA under ambient light as it migrates through the gel; stop the run when desired separation is reached; safely view the cassette on the lighted dock without eye protection.
– DNA bands separated on FlashGel™ Cassettes are also detectable by UV light and may be photographed using standard gel documentation systems. Use the FlashGel™ Camera for best performance.



DNA bands as viewed during a run on the FlashGel™ Dock.

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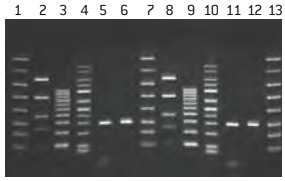
Page 299 (specifications)

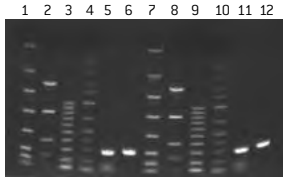
Superior Resolution

– Resolve fragments in 2–7 minutes, and see clean, sharp band separation, and straight, uniform sample lanes

Comparison of FlashGel™ System with Company I



1.2% FlashGel™ Cassette, 12+1-well, single-tier format. 275 V, 7 minute run on The FlashGel™ Dock.



Company I 1.2% gel, 12-well, single-tier format. 30 minute run.

Lanes 1 and 7: FlashGel™ DNA Marker (100 bp–4 kb);
Lanes 2 and 8: FlashGel™ QuantLadder;
Lanes 3 and 9: Lonza 100 bp Ladder;
Lanes 4 and 10: Lonza 50–2500 bp Marker;
Lanes 5 and 11: 285 bp β-actin PCR*;
Lanes 6 and 12: 294 bp control PCR* (Company A)
*Samples diluted with 1X FlashGel™ Loading Dye prior to loading.

www.flashgel.com

Related Products	Page
FlashGel™ System for Recovery	301
FlashGel™ System for RNA	301
FlashGel™ Camera	300
FlashGel™ Dock	300
FlashGel™ Power Supply	300

FlashGel™ System for Recovery

5 Minute DNA Recovery

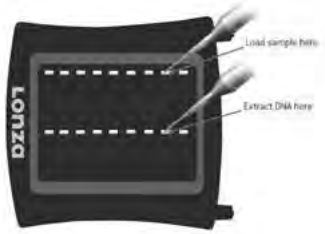
Direct DNA recovery using the FlashGel™ System for Recovery eliminates agarose gel preparation, band excision, and purification. The system delivers highly efficient recovery, free from inhibitors and UV-induced damage, in a simple 5–10 minute protocol.

– Go from sample loading to recovery in just 5 minutes
– Recover samples directly from the cassette, without band excision or purification
– Visualize sample recovery without UV
– Recover at 80%–100% efficiency

www.flashgel.com

Fast, Simple Procedure

1. Load samples in top tier of wells.
2. Run until band of interest almost reaches the second tier of wells.
3. Stop the run and add FlashGel™ Recovery Buffer.
4. Start and run band of interest into the well.
5. Stop the run and remove DNA from well via pipette.



FlashGel™ System for Recovery

Continued

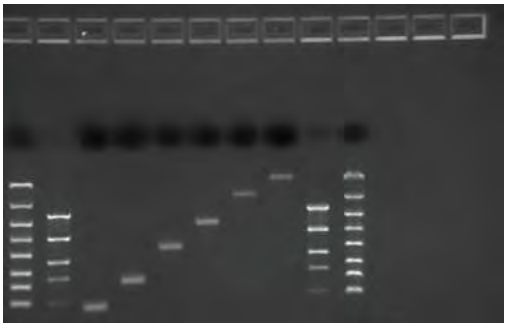
No DNA Damaging UV or Mutagenic Stain Exposure

– Visible light from the compact FlashGel™ Dock illuminates the recovery wells without damage to the DNA or hazard to the user
– The proprietary stain in the FlashGel™ Cassettes enables separation and recovery of very small quantities of DNA, and minimizes user exposure to potential mutagens

Efficient Recovery, Free from Inhibitors

– Samples are recovered at 80%–100% efficiency, are free of inhibitors, and ready for subsequent re-amplification, cloning, or other techniques, without additional clean-up steps

DNA Size Range on the FlashGel™ System for Recovery



Samples were separated and recovered on a FlashGel™ Recovery Cassette. 3 µL aliquots of recovered samples consisting of 100 ng of fragments ranging from 50 bp to 4000 bp separated on a 1.2% FlashGel™ DNA Cassette and compared to the FlashGel™ DNA Marker 100 bp–4 kb and the FlashGel™ QuantLadder.

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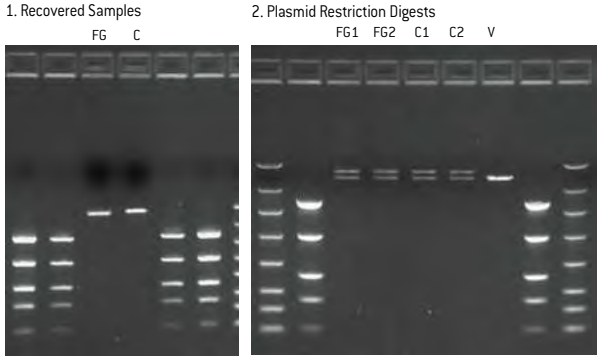
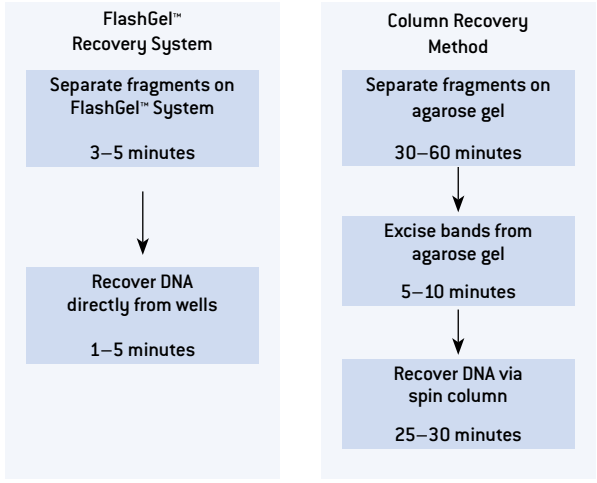
Recovery Efficiency on the FlashGel™ System for Recovery



Plasmid DNA (pBr322) was subjected to restriction enzyme double digestion using *Pst*I and *Bam*HI. Samples of the restricted DNA were separated and 3.2 kb fragments were recovered using the FlashGel™ Recovery System (FG) or spin column kits (C1 and C2). Image 1 compares 5% of each recovered sample. Aliquots of the recovered samples were ligated into *Pst*I/*Bam*HI double digested pUC19 vector (V). Samples of the ligation reactions were transformed into *E.coli* competent cells. The number of colonies obtained with both samples were very similar. Image 2 shows examples of *Pst*I/*Bam*HI cut plasmid samples from two colonies from each sample. V shows a restricted sample of vector with no insert.



Related Products	Page
FlashGel™ System for DNA	301
FlashGel™ System for RNA	301
FlashGel™ Camera	300

FlashGel™ System for RNA

Rapid, Sensitive, Convenient RNA Analysis

The FlashGel™ System for RNA is the ideal tool for rapid verification of sample integrity prior to downstream analysis. High quality, intact RNA is essential for consistent results in gene expression, Northern analysis, cDNA library construction and cDNA labeling for microarrays.

- Get results in 30 minutes or less
- Detect < 10 ng RNA per band
- Avoid hazardous reagents and contaminating RNases

The FlashGel™ System completes RNA analysis in less than 30 minutes and requires < 10 ng total RNA for detection.

Applications

- Verification and analysis of total RNA
- Quick checks of native RNA
- Checking for RNA degradation and mRNA purity

Rapid RNA Analysis Procedure

1. Insert cassette into FlashGel™ Dock.
2. Pre-load wells with RNase-free water.
3. Load samples.
4. Plug in and turn on light and electrophoresis voltage.
5. Run for 8 minutes.
6. Turn off voltage and hold for 10 minutes, or until RNA bands are stained to the desired intensity.
7. Photograph.

Exquisitely Sensitive Detection

- The FlashGel™ System for RNA offers the detection sensitivity of a chip system, without the cost, and rivals the best RNA stains (SYBR® Green and GelStar® Stains), without direct handling of stain solutions. RNA quantities < 10 ng per band are clearly detected on the FlashGel™ System, conserving precious RNA samples

Clean, Enclosed System

- FlashGel™ RNA Cassettes fully enclose the gel, stain and running buffer, eliminating user exposure to hazardous reagents, and protecting samples from contaminating RNases. RNA cassettes are designed for performance and purity, and are guaranteed RNase free. The FlashGel™ Dock provides electrophoresis and visualization of both DNA and RNA cassettes

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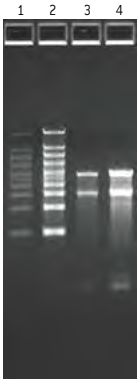
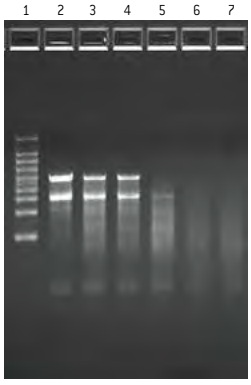
Separation of Total RNA on the FlashGel™ System for RNA



Samples of RNA Marker (Lonza) (lanes 1 and 2) and *E. coli* total RNA (lanes 3 and 4) contain 50 ng (lanes 1 and 3) or 250 ng (lanes 2 and 4) of RNA per 5 µL load. Samples prepared with Formaldehyde Sample Buffer (Lonza) and denatured 5 minutes at 65°C. FlashGel™ RNA Cassette run for 8 minutes, followed by a 20 minute hold prior to imaging.

Checking Sample Quality with the FlashGel™ System for RNA



Sample degradation is visible at low levels on the FlashGel™ System for RNA. FlashGel™ RNA Cassette run for 8 minutes at 225 V, followed by 20 minute hold prior to imaging. Lane 1: RNA marker (Lonza); Lane 2: 250 ng *E. coli* Total RNA; Lanes 3–7: *E. coli* Total RNA incubated with increasing levels of RNase A. Intact, denatured RNA shows sharp, clear bands on the FlashGel™ System. Partially degraded RNA has a smeared appearance, and completely degraded RNA appears as a low molecular weight smear.

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FlashGel™ Camera

From Benchtop to Desktop in 5 Minutes

Capture data from The FlashGel™ System and say goodbye to darkrooms and UV light. Complete separation and documentation safely, at your bench in minutes. This simple digital camera in an enclosed hood connects directly to your laptop or PC via USB. Simply click a button to capture the desired image to a file.

Real time Separation and Documentation

- Complete gel run and image capture in just 5 minutes
- Photograph gels at the bench without UV light

The FlashGel™ Camera Offers

- Sharp, clear high-resolution images
- Simple user interface
- Small, compact design
- Optimized exposure for FlashGel™ Cassettes

Interface of FlashGel™ software with camera. Simply click the camera icon on the dock image to save the gel picture or the printer icon to print.

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Camera Specifications	
Hood dimensions:	10 cm (W) × 11 cm (L) × 16 cm (H)
Camera type:	Digital
Image file type:	.jpg, .tif, .bmp

Related Products	Page
FlashGel™ System for DNA	301
FlashGel™ System for RNA	301
FlashGel™ System for Recovery	301

FlashGel™ Specifications

Simple User Interface Right from Your Laptop or PC

Cassette and Dock Specifications	
Optimal separation and recovery range:	**DNA:** 1.2% agarose: 50 bp–4,000 bp
	DNA: 2.2% agarose: 10 bp–1,000 bp
	RNA: 1.2% agarose: 0.5 kb – 9.0 kb
Separation of fragments >4 kb will be improved by running longer at lower voltage.	
Storage:	**DNA:** Room temperature for 5 months from date of manufacture.
	RNA: Room temperature for 3 months from date of manufacture.
	Shelf life may be extended with refrigerated storage.
Well volume:	12+1-well: 5 µL
	16+1-well: 5 µL
	8+1-well: 12 µL
Gel size:	70 mm (L) × 84 mm (W) × 2 mm (H)
Cassette size:	115 mm (L) × 107 mm (W) × 17 mm (H)
Dock size:	134 mm (L) × 120 mm (W) × 54 mm (H)

FlashGel™ Dock and Cassettes

NOTE: Some components and technology of the FlashGel™ System are sold under licensing agreements. The nucleic acid stain in this product is manufactured and sold under license from Molecular Probes, Inc., and the FlashGel™ Cassette is sold under license from Invitrogen IP Holdings, Inc, and is for use only in research applications or quality control. It is covered by pending and issued patents. The FlashGel™ Dock technology contains Clare Chemical Research, Inc. Dark Reader® transilluminator technology and is covered under US Patents 6,198,107; 6,512,236; and 6,914,250. The electrophoresis technology is licensed from Temple University and is covered under US Patent 6,905,585.

FlashGel™ System Power Supply

Simple, Compact and Powerful

Designed to complement the FlashGel™ Dock, this new power supply has simple program settings and is half the size of other standard power supply units. This 300 volt FlashGel™ Power Supply is capable of powering most standard horizontal and vertical electrophoresis systems.

The FlashGel™ Power Supply offers

- Compact size
- Simple easy-to-use interface
- Multiple jacks to run up to two FlashGel™ Docks at once
- Built-in timer
- Easy to read digital display
- Toggle between volts, current, and time

Physical Specifications	
Terminal Pairs	2 Pairs
Display	3 digit LED
Construction material	Polycarbonate housing and aluminum bottom plates
Unit Dimension	140 × 191 × 84mm
Weight	~1 kg

Electrical Specifications	
Output Voltage / Inc.	10–300V / 1V
Output Current / Inc.	10–400mA / 1mA
Max. Watt	60W
Rated Voltage	100–240 V, 50–60 Hz, 2A
Output Type	Constant Voltage or Constant Current
Control	Microprocessor controller
Timer	1–999 minutes with alarm, continuous
Safety Device	No load detection; shrouded plugs and sockets

Ordering Information – FlashGel™ System

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
FlashGel™ System				
57040	57040	FlashGel™ Camera	Includes: Camera, hood enclosure USB cable and FlashGel™ Capture Software, for use with FlashGel™ Dock	each
57025	57025	FlashGel™ Dock	For use with all FlashGel™ Cassette types	each
50462	50462	FlashGel™ Loading Dye (5X)	Contains xylene cyanol	5 × 1 mL
57067	57067	FlashGel™ System	Includes: FlashGel™ Dock, FlashGel™ Camera, 9 packs FlashGel™ DNA Cassettes (1.2%, 12+1-well single-tier), FlashGel™ Loading Dye and FlashGel™ DNA Marker	each
57068	57068	FlashGel™ Power Supply	For use with all FlashGel™ Cassette types	each
57062	57062	FlashGel™ Device Pack	Includes FlashGel™ Dock, FlashGel™ Power Supply, and FlashGel™ Camera	
57069	57069	FlashGel™ Power Supply Pack	Includes FlashGel™ Dock and FlashGel™ Power Supply	
57065	57065	FlashGel™ Camera Pack	Includes FlashGel™ Dock and FlashGel™ Camera	

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FlashGel™ System – Ordering Information

Continued

Ordering Information – FlashGel™ System

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
FlashGel™ System For DNA				
57063	**57063**	FlashGel™ DNA Kit	Includes FlashGel™ DNA Cassettes (1.2% 12+1-well single tier 9 pack), FlashGel™ Loading Dye, and FlashGel™ Marker 100 bp – 4 kb	Kit
57023	**57023**	FlashGel™ DNA Cassettes – 9 per pack	12+1 single-tier	1.2% agarose, 12+1 single-tier
57029	**57029**	FlashGel™ DNA Cassettes – 9 per pack	16+1 double-tier (34-well)	1.2% agarose, 16+1 double-tier (34-well)
57031	**57031**	FlashGel™ DNA Cassettes – 9 per pack	12+1 single-tier	2.2% agarose, 12+1 single-tier
57032	**57032**	FlashGel™ DNA Cassettes – 9 per pack	16+1 double-tier (34-well)	2.2% agarose, 16+1 double-tier (34-well)
57034	**57034**	FlashGel™ DNA Marker, 100 bp – 3 kb	Ready-to-load, recommended for double-tier cassettes, 100 applications	500 µL
50473	**50473**	FlashGel™ DNA Marker, 100 bp – 4 kb	Ready-to-load, recommended for 1.2% cassettes, 100 applications	500 µL
57033	**57033**	FlashGel™ DNA Marker, 50 bp – 1.5 kb	Ready-to-load, recommended for 2.2% cassettes, 100 applications	500 µL
57026	**57026**	FlashGel™ DNA Starter Kit	Includes FlashGel™ Dock, FlashGel™ Loading Dye, FlashGel™ DNA Cassettes (1.2%, 12+1 well single-tier, 9 packs), FlashGel™ DNA Marker 100 bp–4 kb	each
50462	**50462**	FlashGel™ Loading Dye (5X)	Contains xylene cyanol	5 × 1 mL
50475	**50475**	FlashGel™ QuantLadder, 100 bp (3 ng) – 1.5 kb (30 ng)	Ready-to-load, 50 applications	250 µL
FlashGel™ System for Recovery				
57064	57064	FlashGel™ Recovery Kit	Includes FlashGel™ Recovery Cassettes 1.2% 8+1-well double tier 9 pack, FlashGel™ Recovery Buffer, FlashGel™ Loading Dye FlashGel™ QuantLadder, and Visualization Glasses	Kit
57060	**57060**	FlashGel™ Recovery Buffer	Ready-to-use	2 × 500 µL
57022	**57022**	FlashGel™ Recovery Cassettes – 9 per pack	8+1 double-tier (18-well)	2.2% agarose, 8+1 double-tier (18-well)
57051	**57051**	FlashGel™ Recovery Cassettes – 9 per pack	8+1 double-tier (18-well)	1.2% agarose, 8+1 double-tier (18-well)
57050	**57050**	FlashGel™ Recovery Starter Kit	Includes FlashGel™ Recovery Cassettes (1.2%, 8+1-well double-tier, 9 packs), FlashGel™ Loading Dye, FlashGel™ Recovery Buffer, FlashGel™ QuantLadder, Visualization Glasses, Control Fragment. Dock sold separately.	Kit
57061	**57061**	FlashGel™ Visualization Glasses	For use with all FlashGel™ Cassette types	each
FlashGel™ System for RNA				
57027	**57027**	FlashGel™ Recovery Cassettes–9 per pack	12+1 single-tier, 9 per pack	1.2% agarose, 12+1 single-tier
57028	**57028**	FlashGel™ Recovery Cassettes–9 per pack	16+1 double-tier (34-well), 9 per pack	1.2% agarose, 16+1 double-tier (34-well)
50577	**50577**	FlashGel™ RNA Marker, 0.5 kb–9 kb	Available sizes: 0.5/1.0/1.5/3.0/5.0/9.0 kb	50 µL
57024	**57024**	FlashGel™ System for RNA Starter Pack	Includes FlashGel™ RNA Cassettes 1.2% 12+1-well single tier 9 packs RNA Marker, Sample Buffer, and Molecular Biology Water	Kit
50462	**50462**	FlashGel™ Loading Dye (5X)	Contains xylene cyanol	5 × 1 mL
50475	**50475**	FlashGel™ QuantLadder, 100 bp (3 ng) – 1.5 kb (30 ng)	Ready-to-load, 50 applications	250 µL

NOTE: Due to varying storage requirements, kit components may arrive in separate shipping containers.

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www.flashgel.com

Reliant™ Minigels

Versatile Minigels for Routine DNA Separation and Recovery

MANUFACTURED WITH SeaKem® & NuSieve™ Agarose

Reliant™ Gels are versatile and convenient minigels for nearly any application. Each gel is precision manufactured for rapid and reproducible resolution of DNA sizes from 8 bp to 10 kb. Reliant™ Gels are available in a variety of well formats and agarose concentrations, in TAE and TBE buffer and most are prestained with ethidium bromide.

Benefits

- Manufactured with high quality SeaKem® and NuSieve™ Agarose for reliability
- Compatible with most minigel chambers
- Versatile format options

Applications

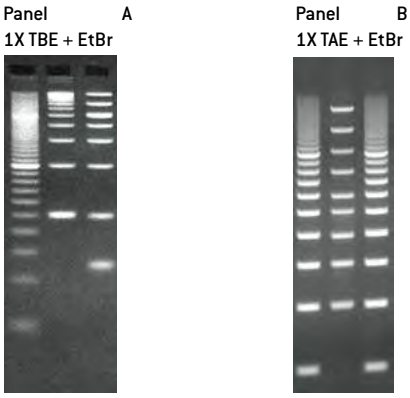
- DNA analysis
- Restriction digests
- Recovery
- PCR and RT-PCR
- Cloning and Blotting

Performance and Quality Tests

- DNase: no activity detected
- Gel performance: sharp bands and low background fluorescence

18°C to 26°C for 6–12 months depending upon agarose concentration

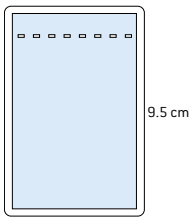
Performance of Reliant™ Minigels



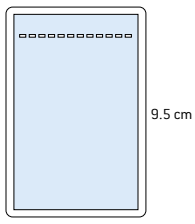
Panel A. 20 bp Ladder (1 µL), 100 bp Ladder (1 µL) and 50–1000 bp marker (2.5 µL) (all Lonza), loaded and run in a 4% NuSieve™ 3:1 Plus Reliant™ Gel containing ethidium bromide. Gel was run at 7 V/cm for 50 minutes using 1X TBE buffer containing 0.5 µg/mL ethidium bromide.
Panel B. A repeating pattern of 500 bp DNA ladder (1 µL/lane) and 1–10 kb DNA marker (2.5 µL/lane) (Lonza) run in a 1% SeaKem® Gold Reliant™ Gel containing ethidium bromide. Gel was run at 5 V/cm for 60 minutes using 1X TAE buffer containing 0.5 µg/mL ethidium bromide.

Specifications	
Gels per box:	20
Gel dimensions:	6.0 cm × 9.5 cm
Gel thickness:	5.5 mm
Tray dimensions:	6.8 cm × 10.2 cm
Well volume:	<15 µL

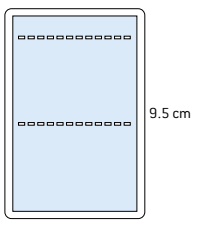
8-well



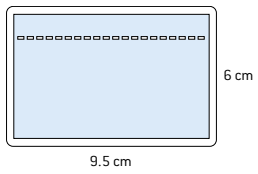
12-well



24-well



20-well



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Reliant™ Minigels

Continued

Ordering Information – SimplyLoad™ Ladders

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Range	Agarose	Size
8-well						
54801	54801	Reliant™ Minigel TAE	No stain	bp 400 ≥ 10,000	1% SeaKem® Gold Plus Agarose	8-well (20 gels/box)
54803	54803	Reliant™ Minigel TAE	With ethidium bromide (0.5 µg/mL)	bp 400 ≥ 10,000	1% SeaKem® Gold Plus Agarose	8-well (20 gels/box)
54903	54903	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 300 ≥ 8,000	1% SeaKem® Gold Plus Agarose	8-well (20 gels/box)
54925	54925	Reliant™ Minigel TAE	With ethidium bromide (0.5 µg/mL)	bp 20 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	8-well (20 gels/box)
54927	54927	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	8-well (20 gels/box)
12-well						
54820	54820	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 300 ≥ 8,000	1% SeaKem® Gold Plus Agarose	12-well (20 gels/box)
54821	54821	Reliant™ Minigel TAE	With ethidium bromide (0.5 µg/mL)	bp 400 ≥ 10,000	1% SeaKem® Gold Plus Agarose	12-well (20 gels/box)
54823	54823	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	12-well (20 gels/box)
54825	54825	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 100 ≥ 3,000	2% SeaKem® Gold Plus Agarose	12-well (20 gels/box)
20-well						
54907	54907	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 300 ≥ 8,000	1% SeaKem® Gold Plus Agarose	20-well (20 gels/box)
54928	54928	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	20-well (20 gels/box)
54938	54938	Reliant™ Minigel TBE	No stain	bp 100 ≥ 3,000	2% SeaKem® Gold Plus Agarose	20-well (20 gels/box)
54939	54939	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 100 ≥ 3,000	2% SeaKem® Gold Plus Agarose	20-well (20 gels/box)
54944	54944	Reliant™ Minigel TBE	No stain	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	20-well (20 gels/box)
24-well						
54813	54813	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 100 ≥ 3,000	2% SeaKem® Gold Plus Agarose	24-well (20 gels/box)
54905	54905	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 300 ≥ 8,000	1% SeaKem® Gold Plus Agarose	24-well (20 gels/box)
54929	54929	Reliant™ Minigel TBE	With ethidium bromide (0.5 µg/mL)	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	24-well (20 gels/box)

Contact Scientific Support to inquire about custom precast gels.

Ordering Information – Supporting Products

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
54945	54945	Reliant™ Gel Reusable UV Transparent Tray	Landscape	each
54946	54946	Reliant™ Gel Reusable UV Transparent Tray	Portrait	each
50655	50655	DNA Loading Buffer (6X)	Ficoll® based with bromophenol blue and xylene cyanol	5 × 1 mL
50836	50836	AccuGENE™ 5X TBE Buffer	0.45 M Tris-borate, 0.01 M EDTA (disodium salt), pH 8.3	20 L
51216	BE51216	AccuGENE™ 50X TAE Buffer	2.0 M Tris-acetate, 0.05 M EDTA, pH 8.3	1 L
50843	BE50843	AccuGENE™ 10X TBE Buffer	0.89 M Tris-borate, 0.02 M EDTA (disodium salt), pH 8.3	1 L
50841	50841	AccuGENE™ 10X TAE Buffer	0.4 M Tris-acetate, 0.01 M EDTA (disodium salt), pH 8.0	4 L
50844	BE50844	AccuGENE™ 10X TAE Buffer	0.4 M Tris-acetate, 0.01 M EDTA (disodium salt), pH 8.0	1 L

Latitude™ HT Gels

Precast Gels for High-throughput Separations

Latitude™ HT Precast Agarose Gels are large format agarose gels designed for high-throughput screening applications. These gels are precision manufactured for rapid, reproducible resolution of DNA sizes from 8 bp to 10 kb. Latitude™ HT Gels are available in multiple well formats (from 100–200 wells) and agarose concentrations, in TAE and TBE buffer, all prestained with ethidium bromide.

Benefits

- Manufactured with high quality SeaKem® or NuSieve™ Agarose for reliability
- Versatile design allows you to run gels in most large submerged electrophoresis systems
- Multichannel pipette compatible

Applications

- High-throughput DNA analysis
- PCR, RT-PCR and Multiplex PCR
- Genotyping
- Fingerprinting
- Library construction

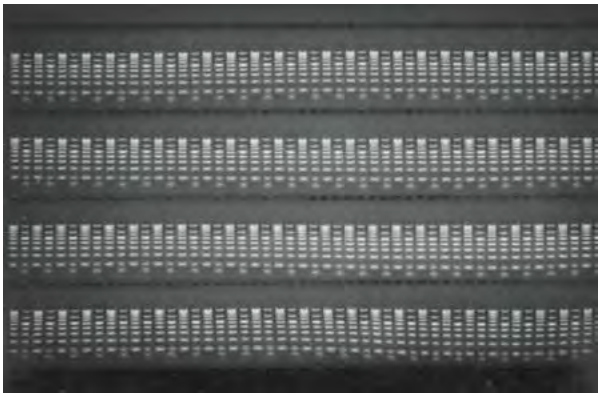
Performance and Quality Tests

- DNase: no activity detected
- Gel performance: sharp bands and low background fluorescence

Chamber Compatibility Information

- Latitude™ HT Gels fit most large submerged electrophoresis systems. Adaptors are available for many nonstandard systems; a complete list can be found on www.lonza.com

Resolution of DNA Markers in a Latitude™ HT Precast Agarose Gel



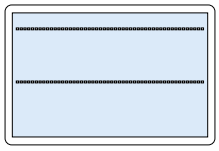
Alternate loads of 50–1000 bp Marker and 100 bp Ladder (Lonza) run in a 2% SeaKem® LE Plus Agarose Gel in 1X TBE buffer containing 0.5 µg/mL ethidium bromide. Gels run at 6 V/cm, 1 hour run using the TruBand™ Anchor.

Specifications	
Gels per box:	5
Gel dimensions:	24 cm × 14 cm
Gel thickness:	6.5 mm
Ethidium bromide:	0.5 µg/mL
Tray dimensions:	25 cm × 15 cm
Well volume:	10 µL–12 µL for 50-well gels 25 µL–30 µL for 25-well gels

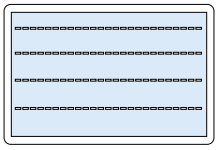
18°C to 26°C for 6–12 months depending upon agarose concentration

www.lonza.com/sourcebook

2 × 50–100-well



4 × 25–100-well



4 × 50–200-well

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

Latitude™ HT Gels

Continued

Ordering Information – Latitude™ HT Precast Gel

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Separation Range	Agarose	Size
57206	57206	Latitude™ HT Precast Gel TAE	Multichannel pipette compatible (alternate well), with ethidium bromide (0.5 µg/mL)	bp 100 ≥ 3,000	2% SeaKem® LE Plus Agarose	2 × 50-wells, 100-well (5 gels/box)
57225	57225	Latitude™ HT Precast Gel TBE	Multichannel pipette compatible (alternate well), with ethidium bromide (0.5 µg/mL)	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	2 × 50-wells, 100-well (5 gels/box)
57226	57226	Latitude™ HT Precast Gel TBE	Multichannel pipette compatible (alternate well), with ethidium bromide (0.5 µg/mL)	bp 100 ≥ 2,000	2% SeaKem® LE Plus Agarose	2 × 50-wells, 100-well (5 gels/box)
57246	57246	Latitude™ HT Precast Gel TBE	Multichannel pipette compatible (consecutive well), with ethidium bromide (0.5 µg/mL)	bp 100 ≥ 2,000	2% SeaKem® LE Plus Agarose	4 × 25-wells, 100-well (5 gels/box)
57255	57255	Latitude™ HT Precast Gel TBE	Multichannel pipette compatible (consecutive well), with ethidium bromide (0.5 µg/mL)	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	4 × 25-wells, 100-well (5 gels/box)
57214	57214	Latitude™ HT Precast Gel TAE	Multichannel pipette compatible (alternate well), with ethidium bromide (0.5 µg/mL)	bp 400 ≥ 10,000	1% SeaKem® LE Plus Agarose	4 × 50-wells, 200-well (5 gels/box)
57234	57234	Latitude™ HT Precast Gel TBE	Multichannel pipette compatible (alternate well), with ethidium bromide (0.5 µg/mL)	bp 300 ≥ 8,000	1% SeaKem® LE Plus Agarose	4 × 50-wells, 200-well (5 gels/box)
57235	57235	Latitude™ HT Precast Gel TBE	Multichannel pipette compatible (alternate well), with ethidium bromide (0.5 µg/mL)	bp 8 ≥ 1,000	4% NuSieve™ 3:1 Plus Agarose	4 × 50-wells, 200-well (5 gels/box)
57236	57236	Latitude™ HT Precast Gel TBE	Multichannel pipette compatible (alternate well), with ethidium bromide (0.5 µg/mL)	bp 100 ≥ 2,000	2% SeaKem® LE Plus Agarose	4 × 50-wells, 200-well (5 gels/box)

Contact Scientific Support to inquire about custom precast gels.

Ordering Information – Supporting Products

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
56991	56991	TruBand™ Gel Anchor		For Owl Millipede™, Shelton JSB-96, Fisher SB-2318 chambers
56993	56993	TruBand™ Gel Anchor		Standard chambers
50655	50655	DNA Loading Buffer (6X)	Ficoll® based with bromophenol blue and xylene cyanol	5 × 1 mL
50836	50836	AccuGENE™ 5X TBE Buffer	0.45 M Tris-borate, 0.01 M EDTA (disodium salt), pH 8.3	20 L
51216	BE51216	AccuGENE™ 50X TAE Buffer	2.0 M Tris-acetate, 0.05 M EDTA, pH 8.3	1 L
50843	BE50843	AccuGENE™ 10X TBE Buffer	0.89 M Tris-borate, 0.02 M EDTA (disodium salt), pH 8.3	1 L
50841	50841	AccuGENE™ 10X TAE Buffer	0.4 M Tris-acetate, 0.01 M EDTA (disodium salt), pH 8.0	4 L
50844	BE50844	AccuGENE™ 10X TAE Buffer	0.4 M Tris-acetate, 0.01 M EDTA (disodium salt), pH 8.0	1 L

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Latitude™ Midigels

Versatile Medium-sized Precast Gels

Latitude™ Precast Agarose Midigels are designed for high sample throughput DNA analysis applications requiring increased resolution distance. These gels are precision manufactured for rapid and reproducible resolution of DNA sizes from 8 bp to 10 kb. Latitude™ Gels are available in a variety of well formats and agarose concentrations, in TAE and TBE buffer.

Benefits

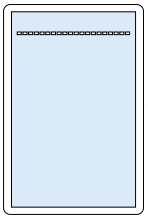
- Manufactured with high quality SeaKem® or NuSieve™ Agarose for reliability
- Latitude™ Gels fit most midigel chambers and provide optimal performance in the Latitude™ Chamber

Performance and Quality Tests

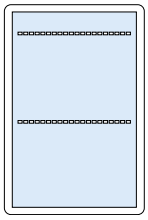
- DNase: No activity detected
- Gel performance: Sharp bands and low background fluorescence

18°C to 26°C for 6–12 months depending upon agarose concentration

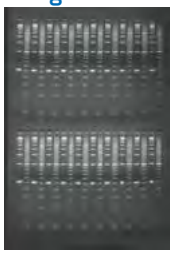
20-well



40-well



Performance of the 40-well Latitude™ Precast Agarose Midigels



Alternate loads of 100 bp DNA ladder and Lonza 20 bp DNA ladder (Lonza) (1 µL marker/lane) run in a 4% NuSieve™ 3:1 Plus Agarose Gel in 1X TBE buffer containing 0.5 µg/mL Ethidium Bromide. 6 V/cm, 70 minute run in a 10 cm × 15 cm Latitude™ Gel Chamber using the TruBand™ Gel Anchor.

Specifications	
Gels per box:	8
Gel dimensions:	10 cm × 15 cm
Gel thickness:	6.0 mm
Ethidium bromide:	0.5 µg/mL
Tray dimensions:	10.4 cm × 15.6 cm
Well volume:	10 µL–12 µL

Ordering Information – Latitude™ Midigel

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Separation Range	Agarose	Size
57200	57200	Latitude™ Midigel TAE	With ethidium bromide (0.5 µg/mL)	bp 400 ≥ 10,000	1% SeaKem® LE Plus Agarose	20-wells (8 gels/box)
57220	57220	Latitude™ Midigel TBE	With ethidium bromide (0.5 µg/mL)	bp 300 ≥ 8,000	1% SeaKem® LE Plus Agarose	20-wells (8 gels/box)
57210	57210	Latitude™ Midigel TAE	With ethidium bromide (0.5 µg/mL)	bp 400 ≥ 10,000	1% SeaKem® LE Plus Agarose	40-wells (8 gels/box)
57211	57211	Latitude™ Midigel TAE	With ethidium bromide (0.5 µg/mL)	bp 100 ≥ 3,000	2% SeaKem® LE Plus Agarose	40-wells (8 gels/box)
57230	57230	Latitude™ Midigel TBE	With ethidium bromide (0.5 µg/mL)	bp 300 ≥ 8,000	1% SeaKem® LE Plus Agarose	40-wells (8 gels/box)
57231	57231	Latitude™ Midigel TBE	With ethidium bromide (0.5 µg/mL)	bp 100 ≥ 2,000	2% SeaKem® LE Plus Agarose	40-wells (8 gels/box)

Ordering Information – Supporting Products

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
56990	56990	Latitude™ Midigel Chamber	Casting accessories not available	Gel chamber
56988	56988	TruBand™ Gel Anchor	Free with your first order of Latitude™ Gels	Latitude™ Chamber
56989	56989	TruBand™ Gel Anchor	Free with your first order of Latitude™ Gels	Standard chambers

PAGEr™ Gold TBE Precast Gels

Polyacrylamide Minigels for DNA Separation

PAGEr™ Gold TBE Precast Gels provide fine resolution of DNA fragments <2,000 bp, and are optimal for resolving 1% differences in DNA fragment size. These ready-to-use gels are specially designed for maximum user convenience. Opening the cassette requires a simple snap of the comb.

Benefits

- Easy-to-load: unique gold colored cassette and marked lanes are easy-to-see
- Easy-to-open: simple snap-open cassette does not require a special opening device

Applications

- Fine resolution of PCR products
- Oligo analysis

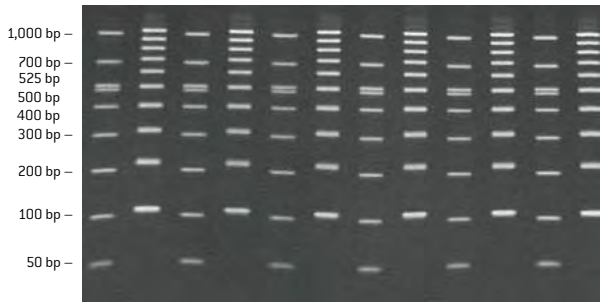
Performance and Quality Tests

- Each lot of PAGEr™ Gold TBE Gels is functionally tested
- Certificate of Analysis available upon request

Separation Ranges for Nucleic Acids in PAGEr™ Gold TBE Gels

Polyacrylamide Concentration	Size Separation Range
6%	75 bp – 2,000 bp
10%	30 bp – 1,000 bp
4–20%	10 bp – 2,000 bp

Performance of PAGEr™ Gold TBE Gels



Alternating lanes of the 50 bp–1,000 bp DNA marker (2 µL/lane) and 100 bp DNA ladder (1 µL/lane) (Lonza) separated on a 4–20% PAGEr™ Gold TBE Gel. Gel run at 200 V for 60 minutes, stained for 15 minutes in 0.5 µg/mL EtBr, and destained for 5 minutes.

Specifications	
Gels per box:	10
Gel dimensions:	8.3 cm × 7.1 cm × 0.1 cm 8.3 cm × 8.3 cm × 0.1 cm
Cassette thickness:	0.49 cm (9 cm × 10 cm) 0.55 cm (10 cm × 10 cm)
Cassette dimensions:	9 cm × 10 cm (L × W) 10 cm × 10 cm (L × W)
Well volume:	10-well – 32 µL 12-well – 20 µL 16-well – 14 µL

2°C to 8°C for 3.5 months from date of manufacture

www.lonza.com/sourcebook

Ordering Information – PAGEr™ Gold TBE Gels

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Range	Size
10-well					
58525	58525	PAGEr™ Gold TBE Gels	Gel concentration: 6%, 10-well, 9 cm × 10 cm	75–2000 bp	10 gels/box
58526	58526	PAGEr™ Gold TBE Gels	Gel concentration: 10%, 10-well, cassette size: 9 cm × 10 cm	25–200 kDa	10 gels/box
58527	58527	PAGEr™ Gold TBE Gels	Gel concentration: 4–20% gradient, 10-well, 9 cm × 10 cm	10–2000 bp	10 gels/box
59525	59525	PAGEr™ Gold TBE Gels	Gel concentration: 6%, 10-well, 10 cm × 10 cm	75–2000 bp	10 gels/box
59526	59526	PAGEr™ Gold TBE Gels	Gel concentration: 10%, 10-well, cassette size: 10 cm × 10 cm	25–200 kDa	10 gels/box
59527	59527	PAGEr™ Gold TBE Gels	Gel concentration: 4–20% gradient, 10-well, 10 cm × 10 cm	10–2000 bp	10 gels/box
12-well					
58528	58528	PAGEr™ Gold TBE Gels	Gel concentration: 6%, 12-well, 9 cm × 10 cm	75–2000 bp	10 gels/box
58530	58530	PAGEr™ Gold TBE Gels	Gel concentration: 4–20% gradient, 12-well, 9 cm × 10 cm	10–2000 bp	10 gels/box
59528	59528	PAGEr™ Gold TBE Gels	Gel concentration: 6%, 12-well, 10 cm × 10 cm	75–2000 bp	10 gels/box
59529	59529	PAGEr™ Gold TBE Gels	Gel concentration: 10%, 12-well, cassette size: 10 cm × 10 cm	25–200 kDa	10 gels/box
59530	59530	PAGEr™ Gold TBE Gels	Gel concentration: 4–20% gradient, 12-well, 10 cm × 10 cm	10–2000 bp	10 gels/box
16-well					
58532	58532	PAGEr™ Gold TBE Gels	Gel concentration: 10%, 16-well, cassette size: 9 cm × 10 cm	30–1000 bp	10 gels/box
58533	58533	PAGEr™ Gold TBE Gels	Gel concentration: 4–20% gradient, 16-well, 10 cm × 10 cm	10–2000 bp	10 gels/box
59531	59531	PAGEr™ Gold TBE Gels	Gel concentration: 6%, 16-well, 10 cm × 10 cm	75–2000 bp	10 gels/box
59532	59532	PAGEr™ Gold TBE Gels	Gel concentration: 10%, 16-well, cassette size: 10 cm × 10 cm	25–200 kDa	10 gels/box
59533	59533	PAGEr™ Gold TBE Gels	Gel concentration: 4–20% gradient, 16-well, 10 cm × 10 cm	10–2000 bp	10 gels/box

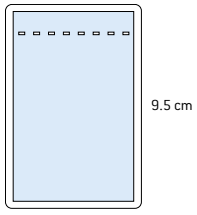
Precast Gels and Related Products for RNA Analysis

Clean, Reliable, Guaranteed RNase-free

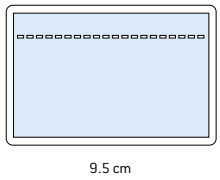
Reliant™ Precast RNA Gels

Versatile, convenient gel options for verification of RNA integrity, Northern blotting, and analysis of RNA transcripts. Reliant™ Precast RNA Agarose Gels are precision cast in 1.25% SeaKem® Gold Agarose with MOPS buffer and are guaranteed RNase-free. Our RNA markers, stains, and buffers are designed to optimize RNA separations.

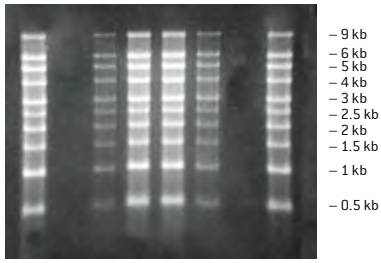
8-well



20-well



Resolution of RNA Markers Run in a Reliant™ RNA Gel



Gel loaded with samples of RNA marker 0.5 kb–9 kb. Marker loaded at 200 ng (lanes 3 and 6) and 1 µg (lanes 1, 4, 5 and 8). Gel run at 5 V/cm for 2 hours using AccuGENE™ MOPS Buffer (1X). RNA stained for 30 minutes using GelStar® Nucleic Acid Gel Stain (1:10,000 dilution).

Benefits

- Guaranteed RNase free
- Compatible with many popular chambers

Applications

- Northern blotting
- RNA integrity checks

Performance and Quality Tests

- Agarose: No RNase activity detected
- Gel performance: Sharp RNA bands and low background with ethidium bromide, SYBR® Green II and GelStar® Nucleic Acid Gel Stains

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Ordering Information – Reliant™ RNA Gell System

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
54922	54922	Reliant™ RNA Gel System	1.25% SKG, MOPS, no stain, cassette size: 6 cm × 9.5 cm, 8-well	20 gels
54948	54948	Reliant™ RNA Gel System	1.25% SKG, MOPS, no stain, cassette size: 9.5 x 6 cm, 20-well	20 gels

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

Precast Gels and Related Products for RNA Analysis

Continued

Sample Buffers

Ready-to-use buffers for denaturation of RNA samples for electrophoresis on Reliant™ and Latitude™ Precast RNA Gels. Ideal for Northern blotting.

Glyoxal: 18°C to 26°C for 12 months; storage at 4°C will extend the stability to 2 years
Formaldehyde: -20°C for 12 months

Ordering Information – RNA Sample Buffers

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50560	50560	Glyoxal Sample Buffer		1.7 mL
50571	50571	Formaldehyde Sample Buffer	RNA denaturing sample buffer, contains bromophenol blue and xylene cyanol	5 × 1 mL

AccuGENE™ 10X MOPS Buffer

Specially formulated MOPS Buffer for use with Latitude™ and Reliant™ Precast Gels. Manufactured with the same reagents used in our precast gels. Buffer contains 0.2 M MOPS (free acid), 0.05 M sodium acetate, 0.01 M EDTA (disodium salt), and 0.01 M EGTA (free acid), pH 7.0.

18°C to 24°C

Ordering Information – AccuGENE™ 10X MOPS Buffer

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
50876	50876	AccuGENE™ 10X MOPS Buffer	0.2 M MOPS (free acid), 0.05 M sodium acetate, 0.01 M EDTA (disodium salt), 0.01 M EGTA (free acid), pH 7.0. No detectable RNase activity	18°C to 24°C	1 L

RNA Marker 0.5–9 kb

RNA Markers 0.5–9 kb suitable for sizing single stranded RNA in glyoxal or formaldehyde denaturing systems. RNA marker consists of ten RNA transcripts: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 9 kb in length. Markers can be denatured with standard procedures, and visualized on Northern blots with labeled lambda sequence. Detect 4 µg with ethidium bromide, or smaller quantities with GelStar® or SYBR® Green II Gel Stains.

-80°C for 24 months or -20°C for 6 months

Page 312–313 (detailed marker sizes)

www.lonza.com/sourcebook

Ordering Information – FlashGel™ RNA Marker

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50575	50575	FlashGel™ RNA Marker	RNA Marker (0.5 to 9 kb) 250 µL (50 µg)	250 µL

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More Precast Gels and Related Products on the next page.

GelStar® Nucleic Acid Gel Stain

GelStar® Nucleic Acid Gel Stain is a fast-acting, fluorescent stain that is up to 15 times more sensitive than Ethidium Bromide for RNA detection.

– Detects 3 ng of RNA or 20 pg of dsDNA

Page 314 (detailed product information)

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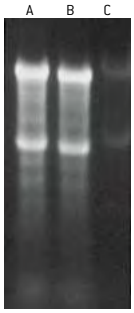
RNA Detection with GelStar® Stain



Samples of *E. coli* total RNA were denatured using the following denaturants: Lane A: Formaldehyde/ Formamide; Lane B: Formamide; Lane C: Glyoxal. Samples were loaded at 2 µg/lane for the formaldehyde/formamide and formamide only denatured samples, and 4 µg/lane for the glyoxal denatured samples. Reliant™ RNA Precast Agarose Gels were run at 7 V/cm for 40 minutes in 1X MOPS Buffer and post stained with GelStar® Gel Stain and photographed on the Clare Chemical Research, Inc., Dark Reader® Transilluminator.

Ordering Information – GelStar® Nucleic Acid Gel Stain 10,000X

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
50535	50535	GelStar® Nucleic Acid Gel Stain 10,000X	Supplied as a 10,000X concentrated solution in DMSO	-20°C	2 × 250 µL
50536	50536	SYBR® Green Gel Stain Photographic Filter	Wratten® #9	18°C to 26°C	3 inch square

Product licensed from Molecular Probes, Inc.

SYBR® Green II Nucleic Acid Gel Stain

SYBR® Green II Nucleic Acid Gel Stain is a highly sensitive fluorescent stain that is ideal for detection of RNA.

– Detects 2 ng of RNA or 100 pg of dsDNA

Page 315 (detailed product information)

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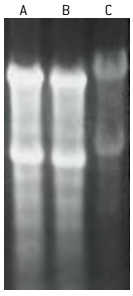
RNA Detection with SYBR® Green II Stain



Samples of *E. coli* total RNA were denatured using the following denaturants: Lane A: Formaldehyde/ Formamide; Lane B: Formamide; Lane C: Glyoxal. Samples were loaded at 2 µg/lane for the formaldehyde/ formamide and formamide only denatured samples, and 4 µg/lane f or the glyoxal denatured samples. Reliant™ RNA Precast Agarose Gels were run at 7 V/cm for 40 minutes in 1X MOPS Buffer and post stained with SYBR® Green II Gel Stain and photographed on the Clare Chemical Research, Inc., Dark Reader® Transilluminator.

Ordering Information – SYBR® Green II Nucleic Acid Gel Stain

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
50522	50522	SYBR® Green II Nucleic Acid Gel Stain	Supplied as a 10,000X concentrated solution in DMSO	-20°C	2 × 500 µL
50523	50523	SYBR® Green II Nucleic Acid Gel Stain	Supplied as a 10,000X concentrated solution in DMSO	-20°C	10 × 50 µL
50530	50530	SYBR® Green Gel Stain Photographic Filter	Wratten® #15	18°C to 26°C	3 inch square

Product licensed from Molecular Probes, Inc.

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Markers, Stains and Buffers

Optimal Performance and Convenience

Great performance starts with high quality agarose and gels, but for complete assurance, you need to use high quality markers, ladders, stains, and buffers. We support a broad offering of products that complement and match the performance of our agarose and precast gels.

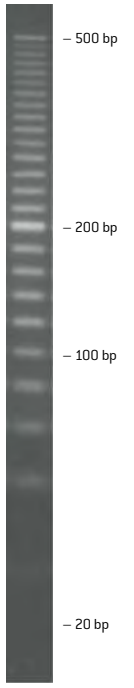
Rapidly estimating fragment size requires clear sharp banding patterns on each and every gel. We offer two types of ladders and markers: Standard and SimplyLoad™. Standard markers and ladders are ready to dilute prior to loading your gel, while our convenient SimplyLoad™ Ladders are premixed, ready for direct loading. Our DNA quantitation ladders are ideal for the accurate estimation of molecular mass of fragments from 10 ng to 100 ng.

Seeing all of your data is critical to the overall success of your experiment. GelStar® Nucleic Acid Gel Stain clearly detects fragments down to 20 pg of DNA. Maximize your performance by adding the stain directly to your gel prior to casting or post-stain your gel. We also offer SYBR® Green Nucleic Acid Gel Stains.

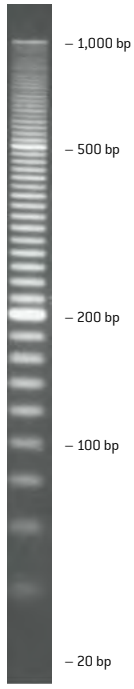
Finally, we offer a complete line of AccuGENE™ Electrophoresis and Molecular Biology Buffers to support your research. Our AccuGENE™ Buffers are formulated to optimize performance of our agarose and precast gel products.

DNA Ladders and Markers

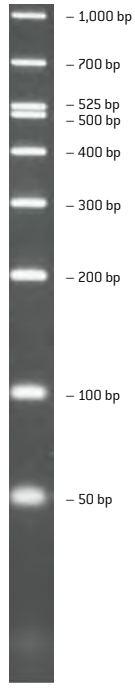
Sizing Made Easy



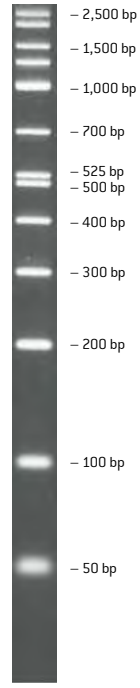
20 bp



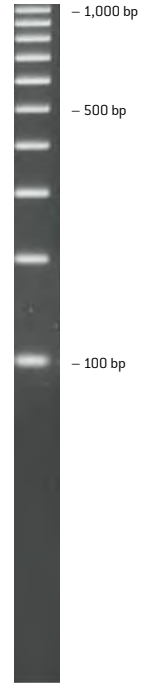
20 bp Ext



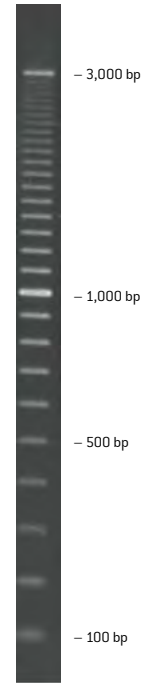
50–1,000 bp



50–2,500 bp

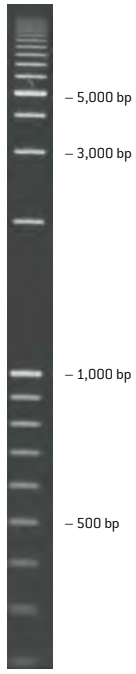


100 bp



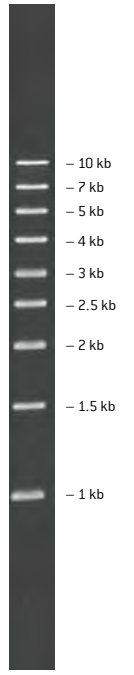
100 bp Ext.

Cat. No.						
	Standard	50330	50320	50461	50631	50321	50322
	SimplyLoad™ Ladders	50331	50326	n/a	n/a	50327	50328



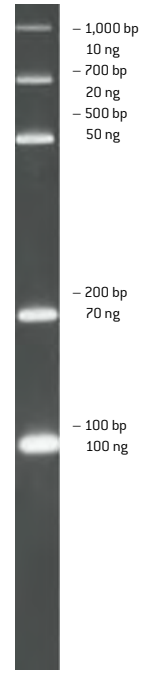
Tandem

500 bp

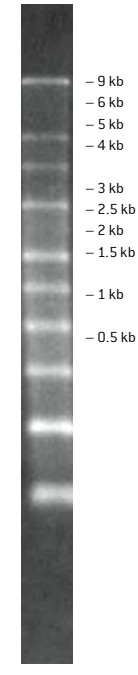


1–10 kb

QuantLadder



Reverse QuantLadder



RNA ladder

Cat. No.						
	Standard	n/a	50323	50471	50334	50335	50575
	SimplyLoad™ Ladders	50333	50329	n/a	n/a	n/a	n/a

SimplyLoad™ Ladders are supplied in ready-to-load concentrations.

Page 462 (detailed band size information)

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DNA Ladders and Markers

Continued

Standard Ladders and Markers are ready-to-dilute prior to loading on your gel. Plasmid-free to ensure minimal background.

SimplyLoad™ Ladders are supplied ready-to-load on your gel. No need for mixing, heating or diluting prior to loading. Plasmid-free to ensure minimal background.

Standard Ladders and Markers: 4°C or -20°C
SimplyLoad™ Ladders: 4°C

Ordering Information – Standard Ladders and Markers

Cat. No. NA	Cat. No. EU	Product Name	Range	Applications	Size
Standard Ladders					
50320	50320	20 bp Extended Range DNA Ladder	20 bp – 1,000 bp	100	150 µL
50321	50321	100 bp DNA Ladder	100 bp – 1,000 bp	100	160 µL
50322	50322	100 bp Extended Range DNA Ladder	100 bp – 3,000 bp	100	150 µL
50323	50323	500 bp DNA Ladder	500 bp – 8,000 bp	200	300 µL
50330	50330	20 bp DNA Ladder	20 bp – 500 bp	100	150 µL
Standard Quantiation Ladders					
50334	50334	DNA QuantLadder	100 bp – 1,000 bp	50	125 µL
50335	50335	DNA Reverse QuantLadder	100 bp – 1,000 bp	50	125 µL
Standard DNA Ladders					
50461	50461	50 bp DNA Marker	50 bp – 1,000 bp	50	250 µL
50471	50471	1kb DNA Marker	1 kb – 10 kb	100	2 × 250 µL
50631	50631	50 bp DNA Marker	50 bp – 2,500 bp	50	250 µL

Ordering Information – SimplyLoad™ Ladders

Cat. No. NA	Cat. No. EU	Product Name	Range	Applications	Size
SimlyLoad™ DNA Ladder					
50326	50326	SimplyLoad™ 20 bp Extended Range DNA Ladder	20 bp – 1,000 bp	100	500 µL
50327	50327	SimplyLoad™ 100 bp DNA Ladder	100 bp – 1,000 bp	100	500 µL
50328	50328	SimplyLoad™ 100 bp Extended Range DNA Ladder	100 bp – 3,000 bp	100	500 µL
50329	50329	SimplyLoad™ 500 bp DNA Ladder	500 bp – 8,000 bp	100	500 µL
50331	50331	SimplyLoad™ 20 bp DNA Ladder	20 bp – 500 bp	100	500 µL
50333	50333	SimplyLoad™ Tandem DNA Ladder	100 bp – 12,000 bp	100	500 µL

Related Products	Page
Agarose	280
Precast Gels	294

GelStar® Nucleic Acid Gel Stain

Exquisitely Sensitive In-gel Stain for DNA and RNA

GelStar® Nucleic Acid Gel Stain is a highly sensitive fluorescent stain for detecting both DNA and RNA. Add GelStar® Stain to your agarose solution prior to casting, or post-stain your gels. GelStar® Stain exhibits exceptional signal-to-noise ratio with minimal background.

Benefits

- **Maximum sensitivity** – Detect as little as 20 pg of dsDNA or 3 ng of RNA
- **Versatile** – Use for agarose or polyacrylamide gel electrophoresis, ideal alternative to silver staining
- **Ultimate user flexibility** – Add GelStar® Stain prior to gel casting or post-stain, no destaining required
- Complete staining solution for all types of nucleic acids
- Detect fragments with either a standard 300 nm UV transilluminator or the Clare Chemical Research, Inc., Dark Reader® Transilluminator

Applications

- DNA and RNA detection
- SSCP and heteroduplex analysis

-20° C for stain
18°C to 26°C for photographic filter

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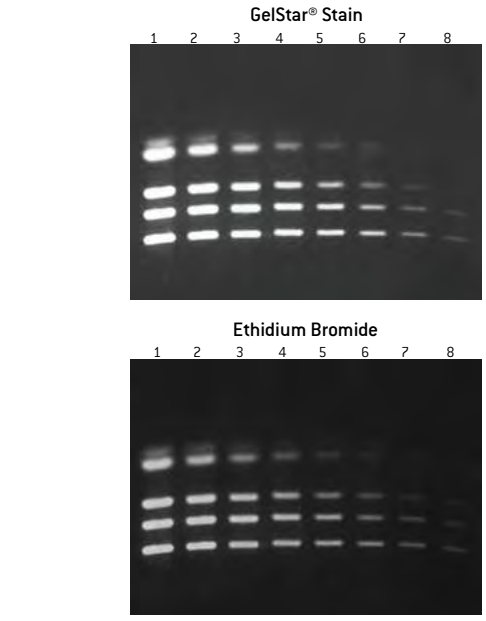
Stain and Method	ssDNA	dsDNA
GelStar® Stain – in gel	25 pg	20 pg
Ethidium bromide, no destain	1.25 ng	350 pg
Ethidium bromide, destain	350 pg	100 pg
SYBR® Green I or II Stain	60 pg	20–30 pg

The FlashGel™ System includes gel cassettes prestained with a similar high-sensitivity stain. Refer to page 295–301

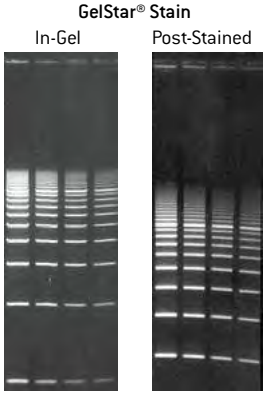
GelStar® Gel Stain Photographic Filter

- Use for optimal sensitivity with black and white film
- Suitable for use with most Polaroid® Documentation or Camera Systems

GelStar® Stain Versus Ethidium Bromide



Serial dilution of SimplyLoad™ DNA QuantLadder on 2% Reliant™ Precast Gels post-stained with 1X GelStar® Stain (top) or 0.5 µg/ml ethidium bromide (bottom) for 45 minutes.



Lonza's 500 bp DNA Ladder was separated on 1% SeaKem® GTG™ Agarose gels 20 cm long, 4 mm thick, run in 1X TBE buffer (Prepared from Lonza's AccuGENE™ 10X TBE Buffer) at 6 V/cm for 3 hours. GelStar® Stain was diluted 1:10,000 and added directly to the agarose or the gel was post stained for 30 minutes in a 1:10,000 dilution of GelStar® Stain in buffer. Lane 1:10 ng DNA/band; Lane 2:5 ng DNA/band; Lane 3: 2.5 ng DNA/band; Lane 4:1.25 ng DNA/band.

Ordering Information – GelStar® Nucleic Acid Gel Stain 10,000X

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
50535	50535	GelStar® Nucleic Acid Gel Stain 10,000X	Supplied as a 10,000X concentrated solution in DMSO	-20°C	2 × 250 µL
50536	50536	SYBR® Green Gel Stain Photographic Filter	Wratten® #9	18°C to 26°C	3 inch square

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Related Products	Page
Agarose	280
DNA Ladders	312-313

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SYBR® Green Nucleic Acid Gel Stains

Sensitive Fluorescent Stains for DNA and RNA

SYBR® Green Nucleic Acid Gel Stains are fluorescent stains for detecting DNA and RNA, exhibiting excellent signal-to-noise ratio with minimal background. SYBR® Green Stains are more sensitive than standard stains, making them convenient alternatives to silver staining and radioisotopes. For maximum detection, gels should be post-stained and photographed with the SYBR® Green Photographic Filter.

SYBR® Green I Stain

- Detects as little as 60 pg of dsDNA and 1 ng oligonucleotides
- Optimal for analysis of PCR products in gels, apoptosis studies, and heteroduplex analysis

SYBR® Green II Stain

- Detects 100 pg of ssDNA and 2 ng of RNA
- Optimal for RNA gel electrophoresis and SSCP analysis

SYBR® Green Gel Stain Photographic Filter

- Required for optimal sensitivity with black and white film
- Suitable for use with most Polaroid® Systems

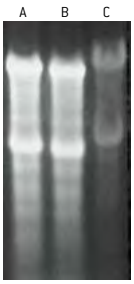
Applications

- DNA and RNA detection
- SSCP and heteroduplex analysis

-20° C for stain
18°C to 26°C for photographic filter

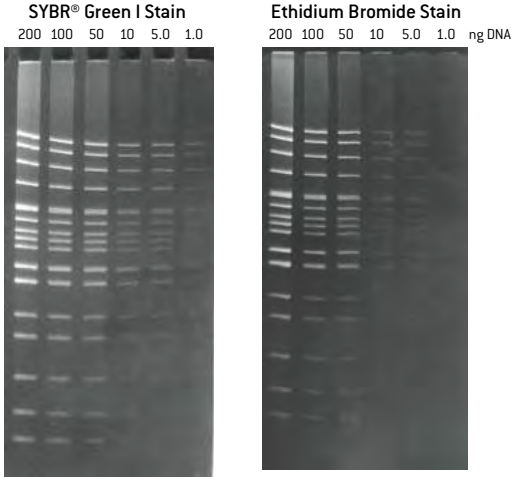
www.lonza.com/sourcebook

RNA Detection with SYBR® Green II Stain



Samples of *E. coli* total RNA were denatured using the following denaturants: Lane A: Formaldehyde/Formamide; Lane B: Formamide; Lane C: Glyoxal. Samples were loaded at 2 µg/lane for the formaldehyde/formamide and formamide only denatured samples, and 4 µg/lane for the glyoxal denatured samples. Reliant™ RNA Precast Agarose Gels were run at 7 V/cm for 40 minutes in 1X MOPS Buffer and post stained with SYBR® Green II Gel Stain and photographed on the Clare Chemical Research, Inc., Dark Reader® Transilluminator.

DNA Stained with SYBR® Green I Stain or Ethidium Bromide



DNA samples (pBR322 *Msp* I digest) ranging from 1 to 200 ng per lane were separated on a 10 cm × 16 cm × 0.1 cm, 4% vertical MetaPhor™ Agarose gel prepared in 1X TBE buffer. The gel was run for 1 hour at 488 V/cm. Following electrophoresis the gel was divided into two, and one half was stained with 1 µg/mL ethidium bromide while the other was stained with SYBR® Green I Stain (1:10,000 dilution of stock). Detection was achieved with standard 300 nm UV transillumination.

Ordering Information – SYBR® Green I Nucleic Acid Stain

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
50513	50513	SYBR® Green I Nucleic Acid Stain	Supplied as a 10,000X concentrated solution in DMSO	-20°C	10 × 50 µL
50512	50512	SYBR® Green I Nucleic Acid Stain	Supplied as a 10,000X concentrated solution in DMSO	-20°C	2 × 500 µL
50523	50523	SYBR® Green II Nucleic Acid Gel Stain	Supplied as a 10,000X concentrated solution in DMSO	-20°C	10 × 50 µL
50522	50522	SYBR® Green II Nucleic Acid Gel Stain	Supplied as a 10,000X concentrated solution in DMSO	-20°C	2 × 500 µL
50530	50530	SYBR® Green Gel Stain Photographic Filter	Wratten® #15	18°C to 26°C	3 inch square

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Related Products	Page
AccuGENE™ Buffers	316
Agarose	280
Precast Gels	294

AccuGENE™ Molecular Biology Buffers

Convenient and Ready-to-use

AccuGENE™ Molecular Biology Buffers are ready-to-use solutions ideal for a wide range of molecular biology applications.

18°C to 24°C

www.lonza.com /sourcebook

Benefits

- **Reliable** – Manufactured according to strict quality control standards to ensure lot-to-lot consistency
- **High quality** – Guaranteed DNase, RNase, and protease-free
- **Efficient** – Ready-made solutions eliminate experiment preparation time
- **Flexible** – Customized solutions are available to meet individual needs

Ordering Information –

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
51200	BE51200	AccuGENE™ Molecular Biology Water		1 L
51223	BE51223	AccuGENE™ Molecular Biology Water		10 L
51224	BE51224	AccuGENE™ Molecular Biology Water		20 L
51201	51201	AccuGENE™ 0.5 M EDTA Solution	Disodium salt, pH 8.0	100 mL
51234	51234	AccuGENE™ 0.5 M EDTA Solution	Disodium salt, pH 8.0	1 L
51202	51202	AccuGENE™ 5 M Sodium Chloride		1 L
51206	51206	AccuGENE™ 10% SDS	Monosodium salt	100 mL
51213	51213	AccuGENE™ 10% SDS	Monosodium salt	500 mL
51203	51203	AccuGENE™ 3 M Sodium Acetate	pH 5.2	500 mL
51205	BE51205	AccuGENE™ 20X SSC Buffer	3.0 M NaCl, 0.3 M sodium citrate, pH 7.0	1 L
51214	BE51214	AccuGENE™ 20X SSPE Buffer	3.0 M NaCl, 0.2 M NaH_2PO_4, H_20, 0.02 M EDTA, pH 7.4	1 L
51235	51235	AccuGENE™ 1X TE Buffer	0.01 M Tris, 0.001 M EDTA (disodium salt), pH 7.4	500 mL
51236	51236	AccuGENE™ 1 M Tris HCl Buffer	pH 7.2	1 L
51237	51237	AccuGENE™ 1 M Tris HCl Buffer	pH 7.4	1 L
51238	51238	AccuGENE™ 1 M Tris HCl Buffer	pH 8.0	1 L
51217	51217	AccuGENE™ LB Broth (Luria Bertani Medium)	10 g/L Bacto-Tryptone, 5 g/L Bacto-Yeast Extract, and 10 g/L NaCl	500 mL
51225	51225	AccuGENE™ 1X PBS	1.7 mM KH_2PO_4, 5 mM NaH_2PO_4, 150 mM NaCl, pH 7.4	1 L
51226	51226	AccuGENE™ 10X PBS	0.017 M KH_2PO_4, 0.05 M Na_2HPO_4, 1.5 M NaCl, pH 7.4	1 L
51229	51229	AccuGENE™ Neutralization Solution	1.5 M NaCl, 1.0 M Tris, pH 7.5	1 L

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AccuGENE™ Electrophoresis Buffers

Optimal Performance

AccuGENE™ Electrophoresis Buffers are formulated for maximum performance and convenience, and are optimized for use with our agarose and precast gels.

AccuGENE™ Buffers for DNA, RNA, and protein electrophoresis are prepared with high quality reagents and use 18 megOhm water. Products are filtered using a 0.2-micron filter, and are guaranteed DNase/RNase free.

Benefits

- **Reliable** – Manufactured according to strict quality control standards to ensure lot-to-lot consistency
- **Efficient** – Ready-to-use solutions eliminate experiment preparation time
- **Flexible** – Customized solutions are available to meet individual needs

18°C to 24°C, 4°C for CE Buffer

Ordering Information – AccuGENE™ Buffers

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
Buffers for DNA Electrophoresis				
50844	BE50844	AccuGENE™ 10X TAE Buffer	0.4 M Tris-acetate, 0.01 M EDTA (disodium salt), pH 8.0	1 L
50841	50841	AccuGENE™ 10X TAE Buffer	0.4 M Tris-acetate, 0.01 M EDTA (disodium salt), pH 8.0	4 L
51216	BE51216	AccuGENE™ 50X TAE Buffer	2.0 M Tris-acetate, 0.05 M EDTA, pH 8.3	1 L
50836	50836	AccuGENE™ 5X TBE Buffer	0.45 M Tris-borate, 0.01 M EDTA (disodium salt), pH 8.3	20 L
50843	BE50843	AccuGENE™ 10X TBE Buffer	0.89 M Tris-borate, 0.02 M EDTA (disodium salt), pH 8.3	1 L
Buffers for RNA Electrophoresis				
50876	50876	AccuGENE™ 10X MOPS Buffer	0.2 M MOPS (free acid), 0.05 M sodium acetate, 0.01 M EDTA (disodium salt), 0.01 M EGTA (free acid), pH 7.0. No detectable RNase activity	1 L
Electrophoresis Loading Buffers				
50655	50655	DNA Loading Buffer (6X)	Ficoll® based with bromophenol blue and xylene cyanol	5 × 1 mL
50632	50632	Triple-Dye Loading Buffer (6X)	Contains bromophenol blue, xylene cyanol, and orange G	1.1 mL
Buffers for Protein Electrophoresis				
50879	BE50879	AccuGENE™ 10X Tris-Glycine Buffer	0.25 M Tris base, 1.92 M Glycine	1 L

Related Products	Page
Agarose	280
Precast Gels	294
Protein Electrophoresis Products	322-340

GelBond® Film

Agarose Support Film

GelBond® Film is a transparent, flexible polyester film designed to support agarose gels. Gels cast on GelBond® Film remain permanently attached to the film through electrophoresis or immunodiffusion and all subsequent fixing, staining, destaining, and drying procedures (gels remain flexible after drying). GelBond® Film is available either as precut sheets or rolls.

Benefits

- **Reliable** – Agarose gels cast on GelBond® Film retain their original dimensions during staining and after drying
- **Durable** – Gels, particularly thin ones, are easier to handle during staining, destaining, and drying when supported
- **Convenient** – Gel orientation can be recorded directly on the GelBond® Film prior to casting
  NOTE: Polyester films will not transmit light of less than 310 nm, and will fluoresce at higher wavelengths.

Applications

- Drying and support of agarose gels

18°C to 26°C

Ordering Information – GelBond® Film Sheets and Rolls

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Sheet Size (mm)	Chamber Compatibility
53734	53734	GelBond® Film Sheets	For agarose gels, 0.2 mm thick	85 mm × 100 mm (100 sheets)	
53745	53745	GelBond® Film Sheets	For agarose gels, 0.2 mm thick	110 mm × 125 mm (100 sheets)	
53746	53746	GelBond® Film Sheets	For agarose gels, 0.2 mm thick	100 mm × 150 mm (100 sheets)	Bio-Rad® Wide Mini-Sub® Cell, Bio-Rad® Sub-Cell® (H)
53748	53748	GelBond® Film Sheets	For agarose gels, 0.2 mm thick	110 mm × 205 mm (100 sheets)	
53749	53749	GelBond® Film Sheets	For agarose gels, 0.2 mm thick	160 mm × 180 mm (100 sheets)	Hoefer® SE400, Hoefer® SE600 (V), Bio-Rad PROTEAN® II xi (V)
53759	53759	GelBond® Film Sheets	For agarose gels, 0.2 mm thick	125 mm × 245 mm (100 sheets)	
53761	53761	GelBond® Film Sheets	For agarose gels, 0.2 mm thick	124 mm × 258 mm (100 sheets)	GE Multiphor® (H)
53740	53740	GelBond® Film Rolls	For agarose gels, 0.2 mm thick	102 mm × 16.5 m (roll)	
53750	53750	GelBond® Film Rolls	For agarose gels, 0.2 mm thick	102 mm × 16.5 m (roll)	
53780	53780	GelBond® Film Rolls	For agarose gels, 0.2 mm thick	203 mm × 16.5 m (roll)	

Custom-cut GelBond® Film is available upon special request. Please inquire for pricing and availability.

(H) = Horizontal; (V) = Vertical

Related Products	Page
Agarose	280

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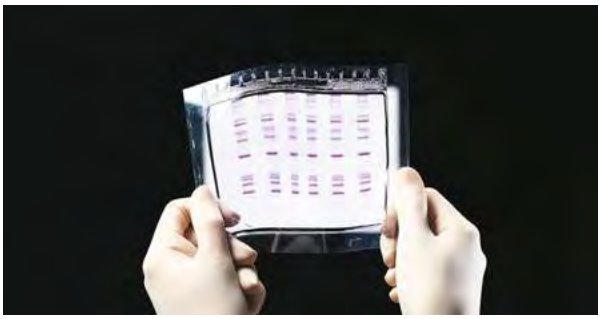
GelBond® PAG Film

Polyacrylamide Support Film

GelBond® PAG Film is a transparent, flexible polyester film designed to support polyacrylamide or MDE™ Gels. The acrylamide monomers covalently attach to the coating on the film during the polymerization reaction. Gels remain permanently attached to the film through electrophoresis and all subsequent fixing, staining, destaining, and drying procedures.



GelBond® PAG Support Film

Benefits

- **Reliable** – Polyacrylamide gels retain their original dimensions during staining and after drying
- **Durable** – Gels, particularly thin ones, are easier to handle during staining, destaining, and drying when supported
- **Convenient** – Gel orientation can be recorded directly on the GelBond® PAG Film prior to casting
  NOTE: Polyester films will not transmit light of less than 310 nm, and will fluoresce at higher wavelengths.

Applications

- Drying and support of polyacrylamide gels

18°C to 26°C, protect from light

Ordering Information – GelBond® Film Sheets and Rolls

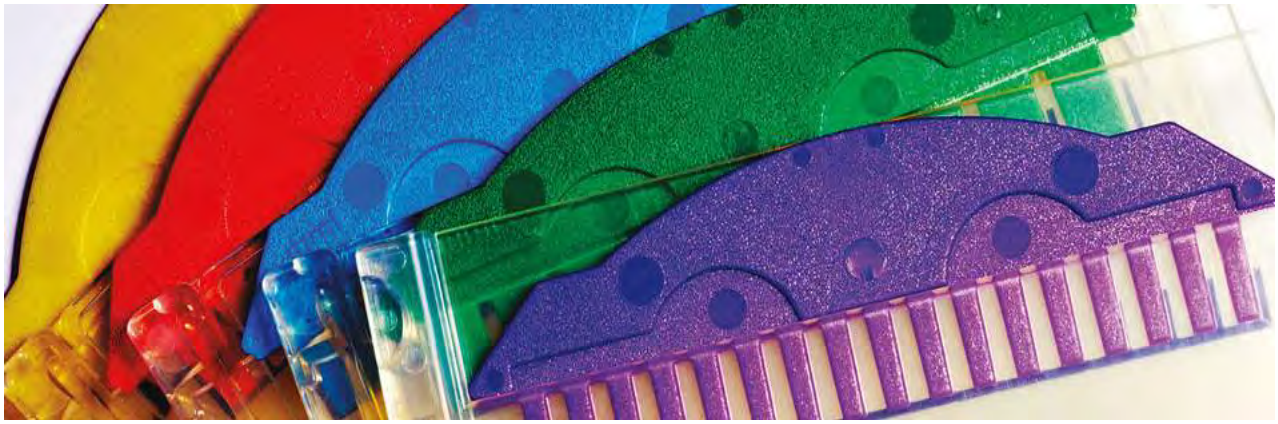
Cat. No. NA	Cat. No. EU	Product Name	Product Description	Sheet Size (mm)	Chamber Compatibility
54711	54711	GelBond® PAG Support Film Sheets	For polyacrylamide gels, 0.2 mm thick	138 mm × 158 mm (50 sheets)	
54723	54723	GelBond® PAG Support Film Sheets	For polyacrylamide gels, 0.2 mm thick	160 mm × 180 mm (50 sheets)	Hoefer® SE400, SE600, Bio-Rad® PROTEAN® II
54727	54727	GelBond® PAG Support Film Sheets	For polyacrylamide gels, 0.2 mm thick	124 mm × 258 mm (50 sheets)	GE Multiphor®
54729	54729	GelBond® PAG Support Film Sheets	For polyacrylamide gels, 0.2 mm thick	220 mm × 165 mm (50 sheets)	
54731	54731	GelBond® PAG Support Film Sheets	For polyacrylamide gels, 0.2 mm thick	199 mm × 264 mm (50 sheets)	
54733	54733	GelBond® PAG Support Film Sheets	For polyacrylamide gels, 0.2 mm thick	203 mm × 260 mm (50 sheets)	GE Multiphor® II
54746	54746	GelBond® PAG Support Film Sheets	For polyacrylamide gels, 0.2 mm thick	350 mm × 430 mm (10 sheets)	X-ray size

Custom-cut GelBond® PAG Support Film is available upon special request. Please inquire for pricing and availability.

Related Products	Page
AccuGENE™ Buffers	316
ProSieve™ Protein Marker	330
SYPRO® Protein Stains	332
ProSieve™ 50 Acrylamide Gel Solution Gels	340

Protein Electrophoresis and Analysis

High-Performance Products that are Fast and Easy to Use



Protein Electrophoresis and Analysis

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North America – Customer Service: **+1 800 638 8174** (toll free); order.us@lonza.com; Scientific Support: **+1 800 521 0390** (toll free) scientific.support@lonza.com

Introduction

Faster Protein Solution for Separations, Blotting and Staining

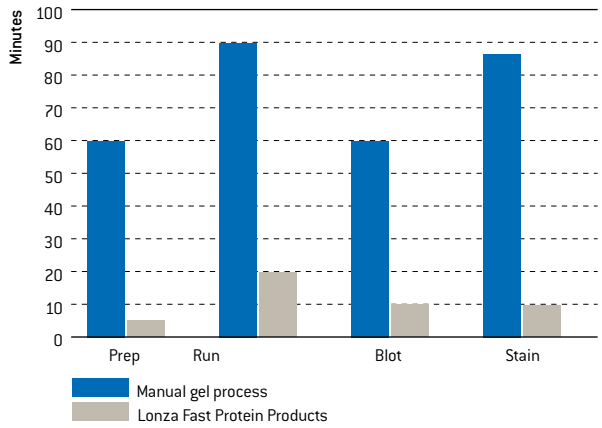
Lonza's new protein solution is addressing the need for a faster, more efficient protein electrophoresis process. Ultimately, these new products combined, take protein separation, western and transfer blotting, and staining from over 5 hours down to less than 1 hour.

Our new PAGEr™ EX Protein Staining Kits and PAGEr™ EX Protein Transfer/Western Blot Kits are demo kits designed to combine the total solution for the ultimate fast separation with staining and transfer in less than 30 minutes.

Each individual component offers a unique solution and can be incorporated into your current protein process:

- PAGEr™ EX Gels were designed for fast 20–25 minute separation, ambient shipping, and are run with using ProSieve™ EX Running Buffer
- ProSieve™ EX Safe Stain takes your staining process down to just 1 step in 10 minutes
- ProSieve™ EX Western Blot Transfer Buffer can be used with most gels for a 10 minute transfer
- ProSieve™ EX Running Buffer offers a reduced separation time for any Tris-glycine gels
- ProSieve™ QuadColor™ Protein Marker provides accurate confirmation of protein transfer in the range of 4.6 kDa–300 kDa

Lonza Protein Solution Time Savings



The time savings and convenience can help your research with each stage of the protein process, from prep to stain or blot time.

PAGEr™ EX Protein Trial Kits

Complete Solution in Less than 1 Hour

The new staining and blotting kits are designed for convenience with everything you need to improve and simplify your protein electrophoresis process.

Kits consist of:

– **PAGEr™ EX Protein Transfer/Western Blotting Kit** – 2 PAGEr™ EX Gels, ProSieve™ EX Running Buffer, ProSieve™ EX Western Blot Transfer Buffer and a ProSieve™ Quad Color Marker

– **PAGEr™ EX Protein Staining Kit** – 2 PAGEr™ EX Gels, ProSieve™ EX Running Buffer, ProSieve™ EX Safe Stain and a ProSieve™ Quad Color Marker

Ordering Information – PAGEr™ Protein Trial Kits

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Range
201747	201747	Fast Protein Transfer Blotting Kit	Mid/high, cassette size: 9 cm × 10 cm, 12-well	25–250 kDa
201742	201742	Fast Protein Transfer Blotting Kit	Mid/high, cassette size: 10 cm × 10 cm, 12-well	25–250 kDa
201743	201743	Fast Protein Transfer Blotting Kit	Low/mid, cassette size: 9 cm × 10 cm, 12-well	25–200 kDa
201744	201744	Fast Protein Transfer Blotting Kit	Low/mid, cassette size: 10 cm × 10 cm, 12-well	25–200 kDa
201745	201745	Fast Protein Staining Kit	Low/mid, cassette size: 9 cm × 10 cm, 12-well	25–200 kDa
201746	201746	Fast Protein Staining Kit	Low/mid, cassette size: 10 cm × 10 cm, 12-well	25–200 kDa
201741	201741	Fast Protein Transfer Blotting Kit	Mid/high, cassette size: 9 cm × 10 cm, 12-well	25–250 kDa
201748	201748	Fast Protein Staining Kit	Mid/high, cassette size: 10 cm × 10 cm, 12-well	25–250 kDa

North America – Customer Service: +1 800 638 8174 (toll free); order.us@lonza.com; Scientific Support: +1 800 521 0390 (toll free) scientific.support@lonza.com

PAGEr™ EX Gels

Redesigned for Speed and Longer Shelf Life

PAGEr™ EX Gels have a proprietary formulation with faster run times and longer shelf life. They cover the full protein size range with fewer configurations making it easier to choose the best one for your needs. They are also compatible with a wide range of chambers. These are more than just another type of protein gel, they are a protein electrophoresis solution.

Benefits

- Fast separation, 20–25 minutes used with ProSieve™ EX Running Buffer
- Reduce your costs with ambient shipping
- 12 month shelf life

Performance and Quality Tests

- Every lot of PAGEr™ EX Gels is functionally tested and 100% guaranteed

2°C to 8°C

PAGEr™ EX Gels Performance and Specifications

Well formats	Size Separation	Equivalent concentration	Cassette Dimensions	Buffer needed	Chambers Types
12-well, 16-well	Low/Med range: 5–225 kDA Med/High range: 10–350 kDA	10% 4–12%	9 × 10 cm, 10 × 10 cm	ProSieve™ EX Running Buffer	See chamber compatibility page 326

Ordering Information – PAGEr™ EX Gels

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Range
12-well				
58722	58722	PAGEr™ EX Gels	Mid/high, cassette size: 9 cm × 10 cm, 12-well	10–350 kDa
59722	59722	PAGEr™ EX Gels	Mid/high, cassette size: 10 cm × 10 cm, 12-well	10–350 kDa
58702	58702	PAGEr™ EX Gels	Low/mid, cassette size: 9 cm × 10 cm, 12-well	5–225 kDa
59702	59702	PAGEr™ EX Gels	Low/mid, cassette size: 10 cm × 10 cm, 12-well	5–225 kDa
16-well				
58724	58724	PAGEr™ EX Gels	Mid/high, cassette size: 9 cm × 10 cm, 16-well	10–350 kDa
59724	59724	PAGEr™ EX Gels	Mid/high, cassette size: 10 cm × 10 cm, 16-well	10–350 kDa
58714	58714	PAGEr™ EX Gels	Low/mid, cassette size: 9 cm × 10 cm, 16-well	5–225 kDa
59714	59714	PAGEr™ EX Gels	Low/mid, cassette size: 10 cm × 10 cm, 16-well	5–225 kDa

ProSieve™ EX Stains

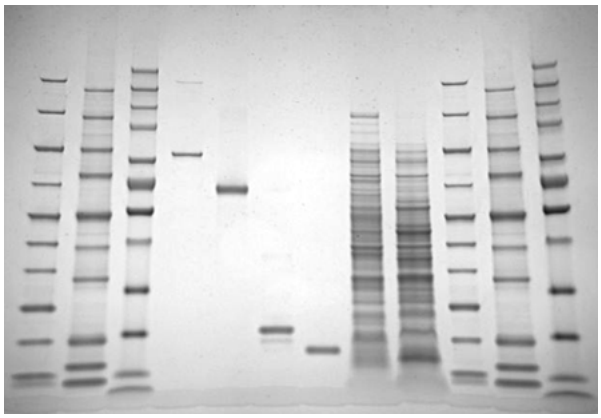
Revolutionary, Fast and Safe

These revolutionary new stains provide faster staining times and less handling than other staining products. With special features that make each product unique, choosing the right stain for your research is easy.

– ProSieve™ EX Safe Stain; the ultimate fast solution that can provide a one step, safe stain in 10 minutes.

2°C to 8°C

ProSieve™ EX Safe Stain with PAGEr™ EX Gels Offers Better Results in Half the Time



15 min gel run with PAGEr™ EX Gels and ProSieve™ EX Running Buffer at 275V, 10 minutes ProSieve™ EX Safe Stain , (total 25 minutes)

Ordering Information – ProSieve™ EX Stains

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
201455	201455	ProSieve™ EX Safe Stain	One step, ten minute protein stain that is non-toxic	1 L
201456	201456	ProSieve™ EX Safe Stain	One step, ten minute protein stain that is non-toxic	25 mL

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ProSieve™ EX Running and Transfer Buffers

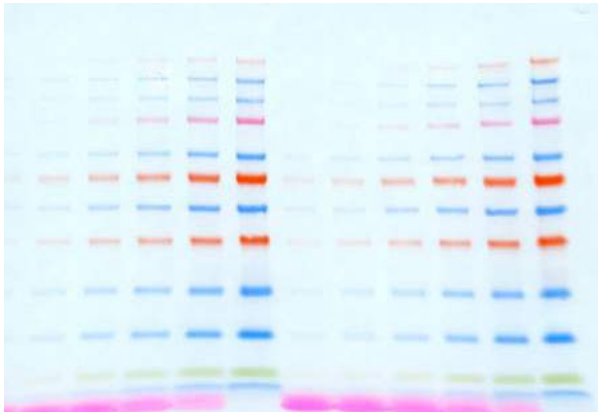
New protein separation and western blot transfer buffers are modified formulations that perform just like tris-glycine, but significantly accelerate run and transfer times without compromising results. Tris-glycine SDS buffers have been recognized as the gold standard for analyzing proteins by PAGE for decades. Now, the standard 2-hour method for protein separation and transfer can be reduced to less than 30 minutes with these buffers:

Benefits

- Separation in 10–20 minutes
- Transfer in 10 minutes
- Compatibility with standard gel systems and protocols
- Razor sharp resolution

2°C to 8°C

ProSieve™ EX Transfer/Western Blot Buffer with PAGEr™ EX Gels Provides Protein Confirmation in Minutes



ProSieve™ EX Transfer/Western Blot Buffer run with PAGEr™ EX Gels and ProSieve™ Running Buffer transferred to PVDF

Ordering Information – ProSieve™ EX Buffers

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
200309	200309	ProSieve™ EX Transfer Buffer	Ten minute protein transfer buffer	1 L
200307	200307	ProSieve™ EX Running Buffer	Less than 30 minute protein running buffer	1 L
200308	200308	ProSieve™ EX Running Buffer	Less than 30 minute protein running buffer	4 L

Related Products	Page
PAGEr™ EX Gels	323
ProSieve™ QuadColor™ Protein Marker	329
ProSieve™ EX Safe Stain	324

PAGEr™ Gold Precast Gels

Reliable, Easy-to-use Minigels

Fresh Gels Every Time

PAGEr™ Precast Gels are easy-to-use protein minigels that offer sharper resolution, more consistent protein transfer, and a long usable shelf life. PAGEr™ Gels are easy-to-use and compatible with most minigel chambers.

Benefits

- **Razor sharp resolution** – Crisp separation of proteins 5 kDa–300 kDa
- **Easy-to-use** – Marked sample lanes for easy loading and simple twist open design
- **Compatible** – Two sizes to fit most chambers
- **Versatile** – Multiple well formats and gel concentrations
- **Tris-Glycine buffer** – Traditional Laemmli separation
- **Fresh** – We ship fresh gels every time for guaranteed performance

Applications

- Western blotting
- Denaturing and native protein electrophoresis
- 2D electrophoresis

Performance and Quality Tests

- Every lot of PAGEr™ Precast Gels is functionally tested and 100% guaranteed

We offer over 70 format options for denatured and native protein separation over a wide molecular weight range, in an array of configurations in both 9 cm × 10 cm and 10 cm × 10 cm sizes to fit popular chambers. See chamber compatibility chart (at right) to determine the right gel size for your system.

Chamber Compatibility

- PAGEr™ Precast Gels are available in 9 cm × 10 cm and 10 cm × 10 cm sizes and fit most standard mini-vertical systems
- Some chambers may require modifications for optimal fit with PAGEr™ Precast Gels

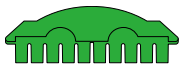
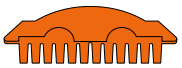
Specifications

Cassette Dimensions	Cassette Thickness	Gel Dimensions
9 cm × 10 cm (L × W)	0.49 cm	7.1 × 8.3 (L × W) × 0.1 cm
10 cm × 10 cm (L × W)	0.55 cm	8.1 × 8.3 (L × W) × 0.1 cm
Gel matrix/buffer	Polyacrylamide/Tris-Glycine **NOTE**: Gels do not contain SDS. Add SDS to sample buffer to create denaturing running conditions.	
Stacking gel	4% stacking gel	
Well formats	2D-well, 8+1-well*, 10-well, 12-well, 16-well, 17-well*	
Cassettes	Plastic	
Storage/shelf life	2°C – 8°C for 3.5 months from date of manufacture Guaranteed 10 weeks shelf life upon receipt	

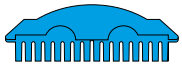
*multichannel pipette compatible well formats

PAGEr™ Precast Gel comb formats

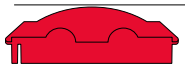
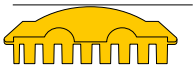
- Comb configurations are designed for a range of sample volumes and throughput, including multichannel pipette compatible formats

Number of wells:	10-well	12-well
Recommended load volume:	32 µL	20 µL



Number of wells:	16-well	17-well*
Recommended load volume:	14 µL	14 µL

Number of wells:	2D-well	8 + 1-well*
Recommended load volume:	550 µL sample, or 7 cm IPG strip, 12 µL marker	30 µL sample 12 µL marker

*Multichannel pipette compatible

Standard Vertical Systems	PAGEr™ Gels
PAGEr™ Minigel Chamber	9 cm × 10 cm or 10 cm × 10 cm gels*
Bio-Rad® Mini-PROTEAN® II, Mini-PROTEAN® 3, Mini-PROTEAN® Tetra, Mini-PROTEAN® Dodeca™ and Ready Gel® Cell Systems.Reverse the inner core gasket so the flat side faces outward.	9 cm × 10 cm gels
Novex® XCell SureLock® Mini-Cell or XCell II Request the spacer for the XCell SureLock® Mini-Cell Chamber from Scientific Support, (Cat. No. 59900).	10 cm × 10 cm gels*
FisherBiotech® Vertical Minigel FBVE121, Owl Separations Systems Wolverine™ P82 Chamber comes with 2 sets of wedges. Use the thinner wedges for the PAGEr™ Gold Gels.	10 cm × 10 cm gels
FisherBiotech® Vertical Minigel FB-VE101, Owl Separations Systems Penguin™ Model P8DS Request adaptor for these chambers from Scientific Support, (Cat. No. 59902).	10 cm × 10 cm gels
Hoefer® Mighty Small™ (SE250, SE260) If using SE250 replace the buffer chamber with a 'Deep lower buffer chamber for the SE260', order number 80–6148–78, from GE Healthcare.	9 cm × 10 cm or 10 cm × 10 cm gels*
Hoefer® Mighty Small™ (SE260)	9 cm × 10 cm or 10 cm × 10 cm gels
EC 120 Mini Vertical Gel System	9 cm × 10 cm or 10 cm × 10 cm gels
CBS Scientific MGV System, (10 cm × 8 cm units)	9 cm × 10 cm gels
Hoefer® Mini VE	10 cm × 10 cm gels

*Recommended for best fit

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Selecting the Best PAGEr™ Gold Precast Gel

Ordering Information – PAGEr™ Gold Gels

Cat. No.	Description	Size
See below	PAGEr™ Gold Tris-Glycine Precast Gels	10 gels per box

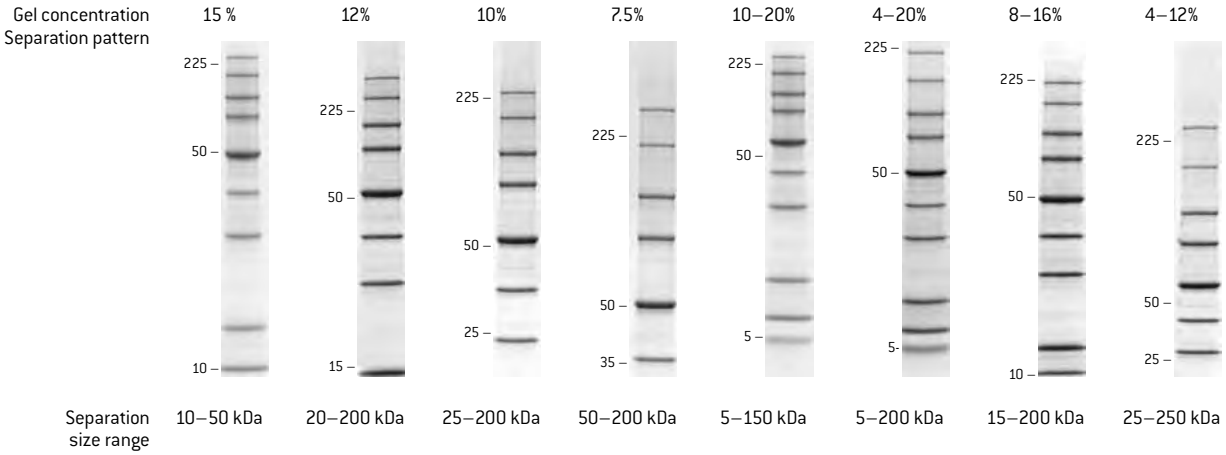
Gel concentration/ separation range	Cassette size (cm)	Cat. No. 20-well	Cat. No. 10-well	Cat. No. 12-well	Cat. No. 16-well	Cat. No. 17-well*	Cat. No. 8 + 1-well*
4–12% gradient 25–250 kDa	9 × 10 10 × 10	— —	58520 59520	58522 59522	58524 59524	— —	— —
4–20% gradient 5–200 kDa	9 × 10 10 × 10	— 59557	58511 59511	58505 59505	58517 59517	58545 59545	58551 59551
8–16% gradient 15–200 kDa	9 × 10 10 × 10	— 59564	58519 59519	58521 59521	58523 59523	58560 59560	58562 59562
10–20% gradient 5–150 kDa	9 × 10 10 × 10	— —	58512 59512	58506 59506	58518 59518	— —	— —
7.5% 50–200 kDa	9 × 10 10 × 10	— —	58507 59507	58501 59501	58513 59513	58540 —	— —
10% 25–200 kDa	9 × 10 10 × 10	— 59554	58508 59508	58502 59502	58514 59514	58542 59542	58548 59548
12% 20–200 kDa	9 × 10 10 × 10	— 59571	58509 59509	58503 59503	58515 59515	58543 59543	— —
15% 10–50 kDa	9 × 10 10 × 10	— 59556	58510 59510	58504 59504	58516 59516	58544 59544	58550 59550

PAGEr™ Gold Scouting Kit

Percentage PAGEr™ Precast Gel

■ **Gel Concentration and Size Separation Range**

– Lower concentrations are best for resolving large molecules and higher concentrations are best for resolving small molecules. Gradient gels are best for proteins that are unknown or occur over a wide molecular weight range.



Gels were run at 175 volts until the dye front reached the bottom of the gel approximately 60 minutes). 8 µL–10 µL of marker was loaded per lane (0.8 µg–1 µg per band). Gels were stained with Coomassie™ Brilliant Blue Stain.

Ordering Information – PAGEr™ Gold Scouting Kit

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
58100	58100	PAGEr™ Gold Scouting Kit	9 cm × 10 cm	Select 6 gels of any type

PAGEr™ Minigel Chamber

Absolute Simplicity and Optimal Performance

PAGEr™ Minigel Chamber

The PAGEr™ Minigel Chamber is designed to provide optimized performance from PAGEr™ Precast Gels and will also work with most other precast minigels. The simple, lock-in-place core design assures a tight, flat fit and eliminates the risk of buffer leaks. No need to remove the core – simply insert gels, close the clamps, fill with buffer and run. Runs one or two gels and accommodates a tank blotting module.

Benefits

- Easy-to-use, lock-in-place core eliminates leaking and minimizes handling
- Perfect fit with 9 cm × 10 cm and 10 cm × 10 cm PAGEr™ Gels
- Even electrical force ensures straight lanes
- Solid, robust construction
- Optimizes performance of PAGEr™ Gels

Applications

- SDS-PAGE electrophoresis
- 2D electrophoresis
- Tank blotting

PAGEr™ Blot Module

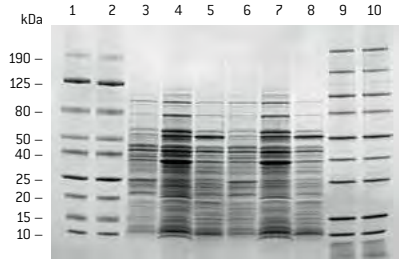
The PAGEr™ Blot Module works directly in the PAGEr™ Minigel Chamber and provides exceptional blotting with a fast, simple protocol.

Benefits

- Color-coded cassettes ensure proper orientation of the gel during transfer
- Transfer time of 90 minutes or less
- Hinged cassette design for easy assembly

The system can be purchased as a kit, including the PAGEr™ Minigel Chamber and PAGEr™ Blot Module, or components may be purchased separately.

Performance of the PAGEr™ Minigel Chamber



Markers and *E. coli* lysate run on a 9 cm × 10 cm PAGEr™ Gel @ 200 V for 60 minutes in the PAGEr™ Minigel Chamber. Samples from left to right: 1 and 2 ProSieve™ Color Protein Marker; 3–8 *E. coli* lysate; 9 and 10 ProSieve™ Protein Marker.

Specifications	
Gel types:	Most standard precast minigels (casting apparatus not included)
Gel sizes:	9 cm × 10 cm (adapter included) and 10 cm × 10 cm
Chamber capacity:	Single gel (blank included), 2 gels, or blot cassettes
Buffer volume:	≈800 mL

www.lonza.com/sourcebook

Ordering Information – PAGEr™ Minigel Chamber

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
59905	59905	PAGEr™ Minigel Chamber		9 cm × 10 cm or 10 cm × 10 cm
59906	59906	PAGEr™ Blot Module		each
59907	59907	PAGEr™ Minigel Chamber and Blot Module Kit	Includes chamber, 2 blotting cassettes, and sponge pads (8/pack).	9 cm × 10 cm or 10 cm × 10 cm

Contact Scientific Support for information about replacement parts.

Related Products	Page
ProSieve™ EX Running and/or Western Blot Transfer Buffer(s)	325
PAGEr™ EX Gels and PAGEr™ Gold Gels	323 & 326
AccuGENE™ Buffers	316

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ProSieve™ Color Protein Markers

Sharp, Accurate Confirmation of Protein Transfer

ProSieve™ Color Protein Markers are ideal for monitoring protein separation prior to staining and provide accurate confirmation of protein transfer in Western blotting.

Benefits

- **Convenient** – Just add water and load (ProSieve™ Color only; not required for ProSieve™ QuadColor™)
- **Sharp** – Multi-colored, readily identifiable band pattern for monitoring electrophoresis and confirming protein transfer
- **Versatile** – Verify protein transfer following Western blotting

ProSieve™ Color Protein Markers are a set of proteins and dyes for use as visible markers in SDS-PAGE gels. During electrophoresis, these markers help monitor the efficiency of separation. In Western blotting, they confirm transfer has occurred from the gel to the membrane. The proteins have been labeled with fluorescent dyes and contain the buffer salts and detergent found in the typical Laemmli buffer system.

ProSieve™ Color Protein Marker, 10–190 kDa

- 9 proteins (10, 15, 20, 25, 40, 50, 80, 125, 190 kDa)

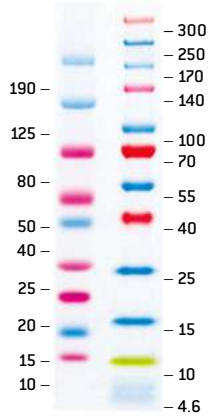
ProSieve™ QuadColor™ Protein Marker, 4.6–300 kDa

- 12 proteins (4.6, 10, 15, 25, 40, 55, 70, 100, 140, 170, 250, 300 kDa)

NOTE: Not recommended for accurate protein sizing. For sharp, accurate sizing, use ProSieve™ Protein Markers (page 328).

-20°C

ProSieve™ Color Protein Markers



Typical Results

ProSieve™ Color Protein Marker Performance vs. Leading Competitors

Markers were run on a Lonza 4–20% PAGEr™ Gold Precast Gel in Tris-Glycine SDS Buffer at 200 V for ~60 minutes.
Lane 1: Bio-Rad® Precision Plus Dual Color Standard
Lane 2: Sigma ColorBurst™ Electrophoresis Marker
Lane 3: Lonza ProSieve™ Color Protein Marker
Lane 4: Lonza ProSieve™ QuadColor™ Protein Marker
Lane 5: Invitrogen BenchMark™ Pre-Stained Ladder
Lane 6: Invitrogen Novex® Sharp Pre-Stained Standard
Lane 7: GE Full Range Rainbow® Marker
Lane 8: Pierce 3-Color Pre-Stained Marker
Lane 9: Lonza ProSieve™ Color Protein Marker
Lane 10: Lonza ProSieve™ QuadColor™ Protein Marker
Lane 11: Invitrogen SeeBlue® Plus 2 Pre-Stained Standard
Lane 12: Bio-Rad® Precision Plus Kaleidoscope™ Standard

Ordering Information – ProSieve™ Color Protein Marker

Cat. No. NA	Cat. No. EU	Product Name	Range	Application	Size
50552	50552	ProSieve™ Color Protein Marker	10 kDa – 190 kDa	10	100 µL
50550	50550	ProSieve™ Color Protein Marker	10 kDa – 190 kDa	50	500 µL
193837	193837	ProSieve™ QuadColor™ Protein Marker	4.6 kDa – 300 kDa	50	500 µL

ProSieve™ Protein Markers

Sharp, Accurate Sizing of Proteins 5 kDa–225 kDa

ProSieve™ Protein Markers consists of a novel set of proteins designed for accurate sizing of protein samples in SDS-PAGE. Markers contain proteins with exact masses and a 50 kDa band of higher intensity for easy identification.

Benefits

- **Simple** – Wide distribution of exact masses simplifies sample determination
- **Accurate** – Recombinant proteins do not contain oligosaccharides that can cause anomalous migration, heterogeneous "fuzzy" bands, and inaccurate size estimation
- **Versatile** – Before Western blotting, markers can be visualized in gel with SYPRO® Tangerine Gel Stain (page 332) without inhibition of protein transfer

-20°C

Ordering Information – ProSieve™ Protein Marker

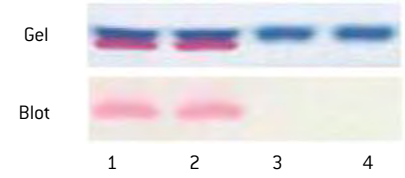
Cat. No. NA	Cat. No. EU	Product Name	Range	Application	Size
193839	193839	ProSieve™ Unstained Protein Marker II	10 kDa – 200 kDa	100	500 µL

ProSieve™ ProTrack™ Dual Color Protein Loading Buffer

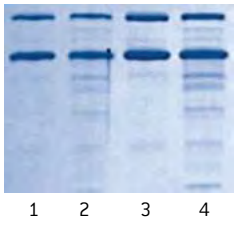
Protect and Track Protein Samples

Benefits

- Protects proteins from degradation during sample preparation
- Two colors for tracking electrophoresis progress (blue) and monitoring Western transfer (pink)
- Contains SDS and DTT for complete protein denaturing



ProSieve™ ProTrack™ Blue Dye monitors protein separation on the gel, while the pink dye confirms transfer of the proteins onto the blot.



Lanes 1 and 3 are proteins protected by ProSieve™ ProTrack™ Dual Color Loading Buffer, Lanes 2 and 4 are proteins prepared and run in a standard loading buffer.

Ordering Information – ProSieve™ ProTrack™ Dual Color Protein Loading Buffer

Cat. No. NA	Cat. No. EU	Product Name	Application	Size
193861	193861	ProSieve™ ProTrack™ Dual Color Protein Loading Buffer (4X)	(4X), 5 µL	5 mL

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AccuGENE™ Protein Electrophoresis Buffers

Optimum Performance

AccuGENE™ Electrophoresis Buffers are formulated to match PAGEr™ Precast Gels. AccuGENE™ Buffers for protein electrophoresis are prepared with high quality reagents and use 18 megOhm water. Products are filtered using a 0.2-micron filter.

18°C to 24°C

www.lonza.com/sourcebook

Benefits

- **Reliable** – Manufactured according to strict quality control standards to ensure lot-to-lot consistency
- **Efficient** – Ready-to-use solutions eliminate preparation time
- **Flexible** – Customized solutions are available to meet individual needs

Ordering Information – AccuGENE™ 10X Tris-Glycine Buffer

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
50879	BE50879	AccuGENE™ 10X Tris-Glycine Buffer	0.25 M Tris base, 1.92 M Glycine	18°C to 24°C	1 L
50882	50882	AccuGENE™ 10X Tris-Glycine SDS Buffer	0.25 M Tris base, 1.92 M Glycine, 1% SDS	18°C to 24°C	4 L

Related Products	Page
PAGEr™ Minigel Chamber	328
PAGEr™ EX Precast Gels	322
PAGEr™ Gold Precast Gels	326
ProSieve™ ProTrack™ Dual Color Protein Loading Buffer	330

SYPRO® Protein Gel Stains

Fast, Sensitive, Easy-to-use Protein Gel Stains

SYPRO® Protein Gel Stains are simple, sensitive alternatives to Coomassie™ Brilliant Blue Stain and Silver Stain for a diverse range of applications from 2D gel staining to staining gels prior to Western blotting.

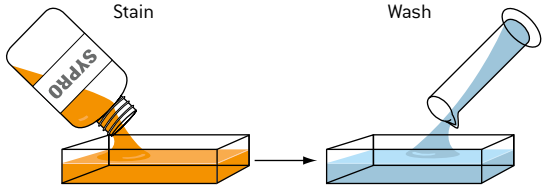
Benefits

- **Exquisitely sensitive** – Detection limits rival the best silver stains
- **Fast and easy** – Simple procedures require no complex fixation or destain
- **Quantitative** – Broad linear range over 3 orders of magnitude
- **Versatile** – Visualize with UV transilluminators, Dark Reader® transilluminators, and laser scanners
- **Compatible** – With downstream processing such as mass spectrometry and microsequencing

Select the Best Stain for Your Application

Application	SYPRO® Ruby	SYPRO® Tangerine	SYPRO® Red
High performance staining	■		■
Staining prior to Western blotting		■	
2D Electrophoresis	■		
Edman microsequencing	■	■	■
Mass spectrometry	■	■	■
Quantitation	■	■	■
Zymography	■	■	■
Electroelution	■	■	■
Membrane staining	■		
Protein expression	■		
Detection prior to Immunostaining	■	■	
Difficult to stain proteins	■		
IEF Gels	■		

Fast, Simple Staining Procedure

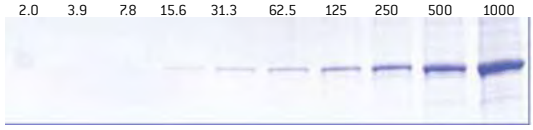


Fixation is required for staining 2D gels in SYPRO® Ruby Gel Stain. No wash step is necessary for SYPRO® Red or Tangerine Gel Stains.

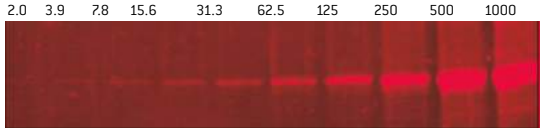
Sensitivity of SYPRO® Stains Compared to Coomassie™ Brilliant Blue and Silver Stain

Serial dilutions of ProSieve™ Protein Marker 50 kDa band on 12% PAGEr™ Gold Precast Gels, stained and photographed as noted. Protein levels indicated in nanograms.

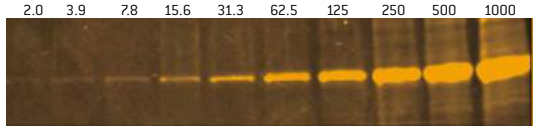
Coomassie™ Brilliant Blue Stain (CCD camera)



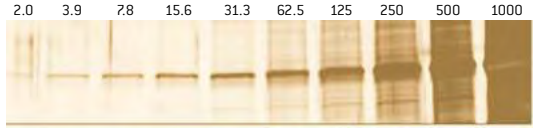
SYPRO® Red Protein Gel Stain diluted in 7.5% acetic acid (Polaroid® Photo UV light)



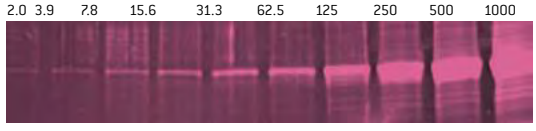
SYPRO® Tangerine Protein Gel Stain diluted in PBS (Polaroid® Photo UV light)



Silver Stain (Amersham PlusOne™ Kit)



SYPRO® Ruby Stain



Related Products	Page
PAGEr™ Precast Gels	326
PAGEr™ EX Gels	323

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SYPRO® Ruby Protein Gel Stain

The Best Stain for 2D Gel Analysis

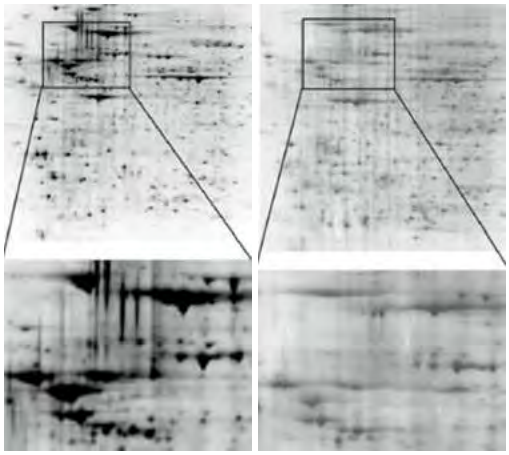
SYPRO® Ruby Protein Gel Stain is a highly sensitive, simple to use fluorescent protein gel stain that can accurately quantitate protein expression levels and is compatible with standard fluorescent visualization systems and downstream identification techniques, such as mass spectrometry.

Benefits

- **Highly sensitive** – Rivals the best silver stain
- **Quantitative** – Broad linear range and consistent gel-to-gel staining
- **Fast** – Simple staining procedure saves time and money
- **High-throughput** – Fast, easy staining of multiple gels
- **Versatile** – Detects difficult to stain proteins

18°C to 26°C

SYPRO® Ruby vs. Silver Stain for 2D Analysis



Proteins from a cell lysate were run on a 2D gel and stained with SYPRO® Ruby Gel Stain (left) or silver stain (right)

Ordering Information – SYPRO® Ruby Protein Gel Stain

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50564	50564	SYPRO® Ruby Protein Gel Stain		200 mL
50562	50562	SYPRO® Ruby Protein Gel Stain	Ready-to-use, single reagent format, stains approximately 20 minigels or 2 large 2D gels.	1 L

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SYPRO® Red Protein Gel Stain

The Fastest, Easiest Stain for Detecting Proteins

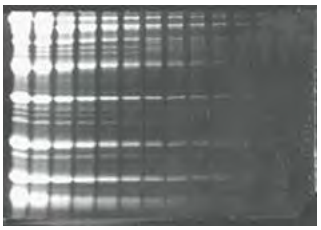
SYPRO® Red Protein Gel Stain is a fast, highly sensitive fluorescent protein gel stain that detects as little as 4 ng–8 ng protein per band.

Benefits

- **Fast** – Complete staining in less than 1 hour
- **Sensitive** – Five times more sensitive than Coomassie™ Brilliant Blue Stain
- **Simple** – No fixation or destaining required
- **Consistent** – Low protein-to-protein variability

Staining is easy – simply soak gels in a solution of 1X SYPRO® Red Stain in 7.5% acetic acid for 40 to 60 minutes. The stain is compatible with UV transilluminators, CCD cameras or laser scanners.
Photographic filters recommended. See page 335.

SYPRO® Red Gel Stain



SDS Polyacrylamide gel stained with SYPRO® Red Gel Stain

18°C to 26°C

Page 465

Ordering Information – SYPRO® Red Protein Gel Stain

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50542	50542	SYPRO® Red Protein Gel Stain	10 × 50 µL as a 5,000X concentrate, sufficient for staining approximately 50 minigels	10 × 50 µL
50543	50543	SYPRO® Red Protein Gel Stain	500 µL as a 5,000X concentrate, sufficient for staining approximately 50 minigels	500 µL

Product licensed from Molecular Probes, Inc

SYPRO® Tangerine Protein Gel Stain

Ideal for Staining Gels Prior to Western Blotting

SYPRO® Tangerine Protein Gel Stain is a versatile, sensitive stain that can be used to visualize proteins prior to Western blotting.

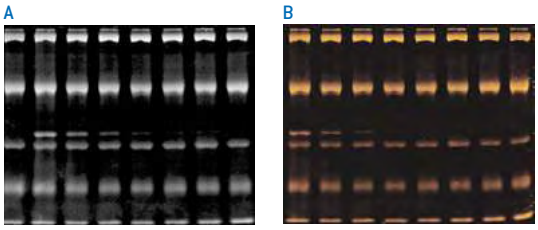
Benefits

- **Visualize proteins prior to transfer** – Does not interfere with protein activity or transfer
- **Safe** – No acids or organic solvents necessary
- **Sensitive** – Detects as little as 4 ng–8 ng protein per band

The staining procedure is fast and simple and does not require the use of organic solvents; staining can be performed in saline or PBS solutions. Proteins can be used in zymography assays or analyzed by mass spectrometry.

18°C to 26°C

Performance of SYPRO® Tangerine Gel Stain



Two identical SDS-PAGE gels were run with samples of protein molecular weight standards (leftmost lanes) and protein molecular weight standards mixed with decreasing amounts of *E. coli* ß-glucuronidase and rabbit liver esterase. Gels were stained for total protein with SYPRO® Tangerine Protein Gel Stain, and for specific enzymatic activities. Both gels were first stained with SYPRO® Tangerine Protein Gel Stain (one gel shown, Panel A). One gel was stained with ELF®-97 ß-d-glucuronidase substrate (E-6587) for the detection of ß-glucuronidase activity (Panel B).

Ordering Information – SYPRO® Tangerine Protein Gel Stain

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50556	50556	SYPRO® Tangerine Protein Gel Stain	Supplied as a 5,000X concentrated solution in DMSO, sufficient for staining 50 minigels	500 µL

Product licensed from Molecular Probes, Inc

SYPRO® Ruby Protein Blot Stain

Fast, Simple, Sensitive Stain for Detecting Proteins on Blots

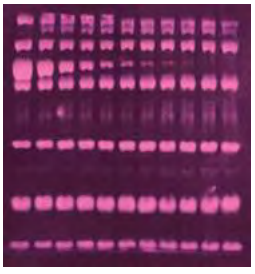
SYPRO® Ruby Protein Blot Stain offers sensitivity levels that rival colloidal stains. The stain is 60-times more sensitive than reversible stains like Ponceau S, and 30-times more sensitive than Amido Black or Coomassie™ Brilliant Blue Stains.

Benefits

- **Highly Sensitive** – Detects as little as 2 ng–8 ng protein per band
- **Fast** – Simple staining procedure takes less than 1 hour
- **Compatible** – With fluorogenic, chemiluminescent and colorimetric detection techniques

18°C to 26°C

Total Protein Detection with SYPRO® Ruby Protein Blot Stain



Molecular weight standards containing decreasing amounts of α-tubulin were run on an SDS-PAGE gel, blotted onto a PVDF membrane and stained with SYPRO® Ruby Protein Blot Stain.

Ordering Information – SYPRO® Ruby Protein Blot Stain

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50565	50565	SYPRO® Ruby Protein Blot Stain		200 mL

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SYPRO® Protein Gel Stain Photographic Filter

For Optimal Detection Sensitivity with Black and White Film Photography

The SYPRO® Protein Gel Stain Photographic Filter is suitable for Polaroid® Camera Systems. The filter does not work with CCD camera systems. Check with the manufacturer for the appropriate filter. Recommended for use with all SYPRO® Protein Gel Stains.

Ordering Information – SYPRO® Protein Gel Stain Photographic Filter

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50540	**50540**	SYPRO® Protein Gel Stain Photographic Filter	Wratten® #9 Gelatin Filter	3 inch square

Related Products	Page
PAGEr™ Minigel Chamber	328
PAGEr™ EX Gels	323
ProSieve™ Protein Marker	330
ProSieve™ ProTrack™ Dual Color Protein Loading Buffer	330
ProSieve™ EX Safe Stain	324

IsoGel™ Agarose and Precast IsoGel™ Agarose IEF Plates

Isoelectric Focusing for Rapid Separation of Large Proteins

Separation of proteins in complex mixtures for analytical resolution can be achieved by isoelectric focusing (IEF), in which proteins are separated based on their net charge (isoelectric point or pI) in the presence of a pH gradient. Agarose has distinct advantages over polyacrylamide gels for isoelectric focusing. Separation in agarose is more rapid, and agarose gels can be used to separate proteins up to 2,000 kDa. We have developed two high quality products that are specifically designed and tested for their performance with IEF.

- IsoGel™ Agarose is a highly purified agarose that is easy to prepare and produces a gel with high clarity and a less restrictive matrix than polyacrylamide
- IsoGel™ Agarose IEF Plates are ready-to-use precast gels supported on GelBond® Film, eliminating gel preparation time and providing easy handling throughout the IEF process

Benefits

- **Safe** – No toxic acrylamide required
- **Fast** – Shorter staining times
- **Simple** – Nontacky and easy to blot

Applications

- Isoelectric focusing
- Antibody separation and analysis
- Immunofixation directly in the gel
- Crossed immunoelectric focusing
- Direct tissue or preparative isoelectric focusing
- Protein blotting
- Immunodetection of proteins

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IsoGel™ Agarose

Highly Purified Agarose for Isoelectric Focusing

Benefits

- **No measurable EEO** – Manufacturing process minimizes fixed anions and mobile cations
- **Versatile** – Sufficiently rigid for casting in vertical tubes (e.g., O'Farrell gels), vertically molded or horizontally open cast thin gels

Applications

- Isoelectric focusing

Reference

O'Farrell, P.H. (1975) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250:** 4007–4021.

Analytical Specifications	
Moisture:	≤10%
Sulfate:	≤0.20%
EEO ($-m_r$):	Not detectable
Gel strength (1.5%):	≥500 g/cm²
IEF test:	Passes test

18°C to 26°C

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Ordering Information – IsoGel™ Agarose

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50202	50202	IsoGel™ Agarose	For use in isoelectric focusing	25 g

Larger package sizes are available upon request. Please inquire for pricing and availability.

Related Products	Page
GelBond® Support Film Sheets	318

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Precast IsoGel™ Agarose IEF Plates

Precast Gels for the Analysis of Antibodies and Proteins up to 2,000 kDa

Benefits

- **Easy handling** – Each gel is supported on GelBond® Film to provide dimensional stability throughout IEF processing
- **Versatile** – Convenient 125 mm × 100 mm gel size fits most horizontal IEF chambers
- **Fast** – Proteins can be quickly transferred from gel to membrane, stained in situ, or detected by antibodies within 1 hour

Applications

- Isoelectric focusing
- Antibody separation and analysis

Performance and Quality Tests

- Each lot of IsoGel™ Agarose IEF Plates is functionally tested; Certificate of Analysis available upon request

2°C to 8°C for 12 months from the date of manufacture
Accessories: 18°C to 26°C

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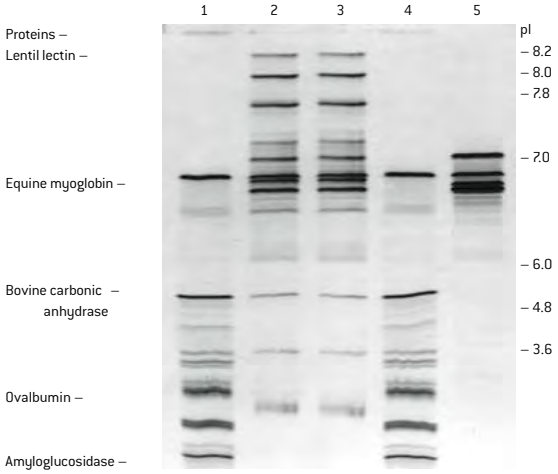
Performance of IsoGel™ Agarose IEF Plate



Separation of proteins in an IsoGel™ Agarose IEF Plate, pH 3–10. Lanes 1 and 4: pI Marker (in-house). Lanes 2 & 3: Broad Range pI 4.45–9.6 marker (Bio-Rad®). Lane 5: Hemoglobin, HB Type AFSC (PE Wallac). 2.5 µL of each sample were loaded on the gel and prefocused at 1 watt for 10 minutes and focused at 2000 volts (max), 25 mA (max), 25 W (max) for 60 minutes on a GE Multiphor® II Chamber at 10°C. The gel was stained with Crowle's stain.

Ordering Information – Precast IsoGel™ Agarose IEF Plates

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Storage Conditions	Size
56015	56015	Precast IsoGel™ Agarose IEF Plates	pH range 3–10	2°C to 8°C	6 plates
56018	56018	Precast IsoGel™ Agarose IEF Plates	pH range 6–10.5	2°C to 8°C	6 plates
56024	56024	Precast IsoGel™ Agarose IEF Plates	pH range 7–11	2°C to 8°C	6 plates
56014	56014	Precast IsoGel™ Agarose IEF Plate, Accessory Pack	Contains masks, 100 mm and 125 mm wicks and blotting paper	18°C to 26°C	Sufficient for 6 plates
56010	56010	Precast IsoGel™ Agarose IEF Plate Accessory Bulk Pack	Contains 125 mm wicks and blotting paper	18°C to 26°C	100 each
56007	56007	Precast IsoGel™ Agarose IEF Blotting Paper		18°C to 26°C	250 sheets

Related Products	Page
GelBond® Support Film Sheets	318

Agarose for Protein Separation

Safe and Easy Separation of Large Proteins and Protein Complexes

Electrophoresis of proteins in agarose gels has distinct advantages compared to polyacrylamide for some applications. Agarose gels can easily and effectively separate high molecular weight proteins and protein complexes (>600 kDa).

Benefits

- **Safe** – No toxic monomer solutions required
- **Efficient recovery** – High recovery yields with simple procedures
- **Flexible** – Gels can be made with standard Laemmli buffer systems

Applications

- Separation of large proteins and protein complexes

18°C to 26°C

www.lonza.com/sourcebook

References

1. **Warren, C.M.** et al. (2003) Vertical agarose gel electrophoresis and electroblotting of high molecular weight proteins, Electrophoresis 24(11): 1695–1702.
2. **Smejkal, G.B.** et al. (2003) Rapid high-resolution electrophoresis of multimeric von Willebrand Factor using a thermopiloted gel apparatus. Electrophoresis 2003, 24: 582–587

Protein Separation

Routine Protein Separation Agarose	Typical Application	Protein Size Range (kDa)	Gel Concentration
MetaPhor™ Agarose	Protein electrophoresis	20–200	4%
MetaPhor™ Agarose	Protein electrophoresis	150–300	3%
MetaPhor™ Agarose	Protein electrophoresis	300–600	2%
SeaKem® Gold Agarose	Protein electrophoresis	600–1,000	1.5%
SeaPlaque™ Agarose	Protein electrophoresis	1,000–5,000	1%

Specialty Protein Separation			
IsoGel™ Agarose	Isoelectric focusing	Separation based on isoelectric point	
SeaKem® HGT Agarose	Counter-immunoelectrophoresis, CIEP, Crossed-IEP		
SeaKem® ME Agarose	Serum protein electrophoresis		
SeaKem® HEEO Agarose	Immunoelectrophoresis of IgG and IgM		
SeaKem® HE Agarose	Serum protein electrophoresis, IEP, Crossed-IEP ,CIEP		

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Agarose for Protein Separation

Continued

Analytical Specifications

	SeaKem® HGT	SeaKem® HE	SeaKem® HEEO	SeaKem® ME
Gelling temp. (1.5%):	42°C ± 1.5°C	36°C ± 1.5°C	36 ± 1.5°C	36 ± 1.5°C
Moisture:	≤10%	≤10%	≤10%	≤10%
Sulfate:	≤0.30%	≤0.20%	≤0.25%	≤0.20%
EEO (–mr):	≤0.10	0.23–0.26	≥0.30	0.16–0.19
Gel strength (1%):	≥800 g/cm^2	≥650 g/cm^2	≥650 g/cm^2	≥1,000 g/cm^2

Ordering Information – Agarose for Protein Separation

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50101	50101	SeaPlaque™ Agarose	A Low melting alternative for separating proteins ≥600 kDa.	25 g
50100	50100	SeaPlaque™ Agarose	A Low melting alternative for separating proteins ≥600 kDa.	125 g
50014	50014	SeaKem® ME Agarose	An ideal alternative to polyacrylamide for serum protein electrophoresis	500 g
50011	50011	SeaKem® ME Agarose	An ideal alternative to polyacrylamide for serum protein electrophoresis	25 g
50010	50010	SeaKem® ME Agarose	An ideal alternative to polyacrylamide for serum protein electrophoresis	125 g
50041	50041	SeaKem® HGT Agarose	High gelling temperature, high clarity agarose for use in counter-immunoelectrophoresis and crossed immunoelectrophoresis	25 g
50040	50040	SeaKem® HGT Agarose	High gelling temperature, high clarity agarose for use in counter-immunoelectrophoresis and crossed immunoelectrophoresis	125 g
50031	50031	SeaKem® HEEO Agarose	A very high EEO agarose useful in applications requiring significant cathodal migration, such as immunoelectrophoresis of IgG and IgM. May also be blended with Lower EEO agarose to achieve a specific EEO value.	25 g
50030	50030	SeaKem® HEEO Agarose	A very high EEO agarose useful in applications requiring significant cathodal migration, such as immunoelectrophoresis of IgG and IgM. May also be blended with Lower EEO agarose to achieve a specific EEO value.	125 g
50021	50021	SeaKem® HE Agarose	A high EEO agarose that provides enhanced resolution in immunoelectrophoresis, crossed immunoelectrophoresis, counter-immunoelectrophoresis, and serum protein electrophoresis.	25 g
50020	50020	SeaKem® HE Agarose	A high EEO agarose that provides enhanced resolution in immunoelectrophoresis, crossed immunoelectrophoresis, counter-immunoelectrophoresis, and serum proteinn electrophoresis.	125 g
50152	50152	SeaKem® Gold Agarose	Effective for separating proteins ≥600 kDa	25 g
50150	50150	SeaKem® Gold Agarose	Effective for separating proteins ≥600 kDa	125 g
50181	50181	MetaPhor™ Agarose	Effective for separating proteins ≥600 kDa	25 g
50180	50180	MetaPhor™ Agarose	Effective for separating proteins ≥600 kDa	125 g
50202	50202	IsoGel™ Agarose	For use in isoelectric focusing	25 g

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Precast IsoGel™ Agarose IEF Plates	337

ProSieve™ 50 Acrylamide Gel Solution

Modified Acrylamide Formulation for High Performance Electrophoresis of Large Proteins

Benefits

- **Gradient separation** – From easy-to-cast single concentration gels
- **Easy-to-handle** – Gels are more durable than standard acrylamide
- **Sharp resolution** – Resolves large proteins (>200 kDa)
- **Fast** – Shorter destaining times and faster protein mobility times
- **Low background** – Even when used with silver stain

Applications

- Protein gel electrophoresis

18°C to 26°C

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www.lonza.com/sourcebook

Ordering Information – ProSieve™ 50 Acrylamide Gel Solution

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
50617	50617	ProSieve™ 50 Acrylamide Gel Solution	50% concentration	125 mL
50618	50618	ProSieve™ 50 Acrylamide Gel Solution	50% concentration	250 mL

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QC Testing Solutions

Endotoxin Detection Assays

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Endotoxin Detection Assays

Endotoxin Detection Assays

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Introduction

Endotoxin Detection: A Brief History

Ever since the pharmaceutical industry began manufacturing injectables, pyrogen detection tests have been an absolute necessity. Pyrogens are substances that can cause fever, shock, and even death if high levels are introduced into the body. Endotoxins are natural compounds found in the outer cell membrane of Gram-negative bacteria and are released upon cell lysis. Endotoxins are a type of pyrogen. Today, endotoxin detection tests are performed on raw materials, in-process materials, and for the final release of pharmaceutical and medical device products.

For most of the 20th century, the rabbit pyrogen test was the standard method of testing for pyrogenicity. This test, which takes approximately four hours, is accomplished by injecting the drug being analyzed into a rabbit's ear. If the animal develops a fever it confirms the presence of pyrogens.

The LAL (Limulus Amebocyte Lysate) test was commercially introduced in the 1970s. LAL is derived from the blood cells, or amebocytes, of the Atlantic horseshoe crab (Limulus polyphemus). LAL was developed into a test for endotoxin after Frederick Bang and Jack Levin observed that the amebocytes of the horseshoe crab contain a clotting agent that forms in the presence of Gram-negative bacteria. They recognized that this clotting agent could be used as a definitive way to test pharmaceutical drugs for the presence of Gram-negative bacteria and their endotoxins. In a notice published in the Federal Register on November 4, 1977, the FDA described conditions for the use of LAL as an end-product test for endotoxin in human biological products and medical devices. The FDA widely recognizes that the LAL test is much faster, more economical, and more efficient than the rabbit pyrogen test. In addition, the LAL test is less labor intensive than the rabbit test, which makes it possible to perform many tests in a single day.

To obtain the lysate required for the LAL test, horseshoe crabs are taken from the ocean floor and a small amount of their blood is drawn. The animals are then returned unharmed to the sea. The crab's blood cells, or amebocytes, are then separated and lysed to obtain the cellular proteins.

As LAL became the preferred endotoxin detection test, different methods were developed. Each method with its own unique benefits. For example, Gel Clot LAL (PYROGENT™) provides a simple positive / negative result and is mentioned in most pharmacopeial monographs as the official referee test. The kinetic turbidimetric LAL assay (PYROGENT™-5000) gives a quantitative result and offers an economical choice for water or large volume parenterals. The endpoint chromogenic LAL test (QCL-1000™) offers a quantitative result and exhibits less product interference than LAL methods utilizing the clotting protein. Detecting as low as 0.005 EU/mL, the kinetic chromogenic LAL assay (Kinetic-QCL™) provides the benefit of less product interference for proteins, vaccines, and other biologicals while also being our most sensitive assay.

Currently the FDA, the United States Pharmacopeia (USP), the European Pharmacopeia (EP), and the Japanese Pharmacopeia (JP) accept all of the above LAL methods, as do most individual country pharmacopeias.

More recently, Lonza scientists have developed a reliable and sustainable endotoxin detection test method that is not derived from horseshoe crab blood. The PyroGene™ Assay is based on the recombinantly expressed Factor C which is the first component in the LAL clotting cascade activated by endotoxin. It is specific for endotoxin and offers a reliable alternative for endotoxin release testing. The PyroGene™ Assay promises to reduce the dependence on animal-based endotoxin assays. In 2009, the FDA approved 510(K) applications that included the PyroGene™ Assay as the final release test. The latest FDA Guidance for Industry document on "Pyrogen and Endotoxins Testing: Questions and Answers" from 2012 accepts the use of PyroGene™ as an alternative method. Please refer to page 354 for further information.

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Overview of LAL Testing Procedures

There are four basic types of assays, each of which is designed to perform a different aspect of LAL testing. Our WinKQCL™ Software supports all of these assay types and is the ideal tool to accompany your quantitative endotoxin assays. It offers a fully integrated solution for reporting and analyzing your endotoxin assay results.

Routine

A routine assay calculates the concentration of endotoxin in unknowns by comparison to the performance of a series of endotoxin standards. As part of a routine assay, the user has the option to include a Positive Product Control (PPC) as a monitor for product inhibition or enhancement. A PPC is a sample of product to which a known amount of endotoxin has been added. For quantitative assays, our WinKQCL™ Software automatically calculates the amount of endotoxin recovered in the PPC and compares it to the known amount of the endotoxin in the well to give the user a percentage of recovery.

Inhibition/Enhancement

The Limulus Amebocyte Lysate reaction is enzyme mediated and, as such, has an optimal pH range, specific salt concentrations, and divalent cation requirements. Occasionally, test samples may alter these optimal conditions to an extent that the lysate is rendered insensitive to endotoxin. Negative results with samples that inhibit the LAL test do not necessarily indicate the absence of endotoxin.

An inhibition/enhancement assay is designed to determine what level of product dilution or other treatment overcomes inhibition or enhancement. Each product dilution must be accompanied by a Positive Product Control (PPC). For quantitative assays, our WinKQCL™ Software calculates the amount of endotoxin recovered in the PPC for comparison to the known amount of endotoxin spike. In this manner it can be determined which product dilutions are non-inhibitory.

RSE/CSE

An RSE/CSE assay is designed to determine the potency of a Control Standard Endotoxin (CSE) in terms of the concentration units of the Reference Standard Endotoxin (RSE). The assay requires a single series of RSE dilutions and one or more sets of dilutions of the CSE. If you buy matched reagents, we have already performed this test for you. Our CSE is matched against the USP RSE. Matched CSE is either part of the kit or is available separately.

Initial Qualification

An Initial Qualification assay is required as part of the validation of the LAL assay and is also to be performed with each new lot of reagents. It serves to confirm reagent performance and assure reproducibility. In addition, it shows analyst qualification. For this assay, a series of endotoxin standards is prepared and tested in at least triplicate. To confirm sensitivity/linearity, the test result must meet regulatory requirements as defined by pharmacopeia. For gel clot assays, the determined end-point must fall between 2 λ and 0.5 λ of the labeled sensitivity. For the quantitative assays, the results are used to generate a standard curve which must have a correlation coefficient of ≥ |0.980|. The Initial Qualification assay does not provide for the inclusion of any samples.

Endotoxin Detection Methods

Qualitative
(Yes/No Answer)

Product: PYROGENT™ Gel Clot LAL Assay

- **Method** – Visual inspection of gel formation
- **Maximum sensitivity** – 0.03 EU/mL
- **Instrument required** – A dry heat block or water bath

Benefits
- Simple LAL test not requiring sophisticated instrumentation and software

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Overview of Endotoxin Detection Methods

Continued

Quantitative

(Results calculated from standard curve)

Product: Kinetic-QCL™ Kinetic Chromogenic LAL Assay

- **Method** – Kinetic measurement of color development
- **Maximum sensitivity** – 0.005 EU/mL
- **Instrument required** – Incubating absorbance reader

Benefits

- Our most sensitive LAL-based method
- Less sensitive to product inhibition
- Ideal for biological products such as vaccines and antibiotics

Product: QCL-1000™ Endpoint Chromogenic LAL Assay

- **Method** – Endpoint measurement of color development
- **Maximum sensitivity** – 0.1 EU/mL
- **Instrument required** – Spectrophotometer or absorbance reader, dry heat block

Benefits

- Results in 16 minutes

Product: PYROGENT™-5000 Kinetic Turbidimetric LAL Assay

- **Method** – Kinetic measurement of turbidity development
- **Maximum sensitivity** – 0.01 EU/mL
- **Instrument required** – Incubating absorbance reader

Benefits

- Cost-effective method for water and large volume parenterals

Product: PyroGene™ rFC Endpoint Fluorescent Assay

- **Method** – Endpoint measurement of fluorescence
- **Maximum sensitivity** – 0.005 EU/mL
- **Instrument required** – Incubating fluorescence reader

Benefits

- Elimination of false positive glucan reactions
- Less lot-to-lot variability
- Animal-free source and security of supply
- FDA acknowledged alternative to LAL

Kinetic Chromogenic LAL Assay Overview

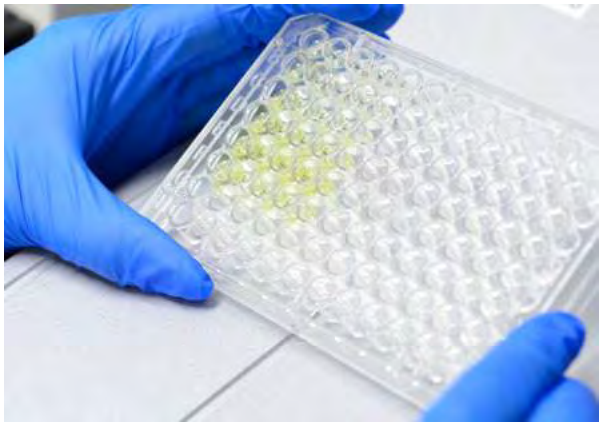
The Kinetic-QCL™ Kinetic Chromogenic Assay is a quantitative, kinetic assay for the detection of Gram-negative bacterial endotoxin. A sample is mixed with the reconstituted LAL reagent in a 96-well plate and placed in an incubating absorbance plate reader that measures absorbance at 405 nm. The reaction is automatically monitored over time for the appearance of a yellow color.

In the presence of endotoxin, the lysate will begin to cleave the chromogenic substrate, causing the solution to become yellow. The time required for the change is inversely proportional to the amount of endotoxin present. The concentration in unknown samples can be calculated from a standard curve. Due to the nature of this assay, the Kinetic-QCL™ Assay is less impacted by inhibitory products that may interfere with the clotting mechanism in turbidimetric and gel clot assays. This feature, along with the sensitivity range of 0.005 to 50 EU/mL, makes this assay optimal for biological products such as vaccines and antibiotics.

Using our extensive experience and practical expertise with endotoxin detection and its regulatory requirements, Lonza has developed an integrated system to support quantitative endotoxin detection. Each system component has been validated and can be verified. This all leads to reliable, reproducible, and accurate quantitative results.

Each quantitative system incorporates three elements:
- Kinetic-QCL™ Kinetic Chromogenic LAL Assay
- WinKQCL™ Endotoxin Detection and Analysis Software
- Incubating Absorbance Plate Reader

These elements integrate seamlessly to meet your testing requirements, providing meaningful results that allow you to be confident in your critical decision-making.



Benefits
- Sensitivity range from 0.005 to 50 EU/mL

Applications
- Ideal for biological products such as vaccines and antibiotics

Requirements
- Incubating Absorbance Plate Reader
- WinKQCL™ Software
- LAL Reagent Water (for larger kits)
- Pyrogen-free Test Tubes
- LAL Reagent Grade Multi-well Plates

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Kinetic-QCL™ Kinetic Chromogenic LAL Assay

The Kinetic-QCL™ Kinetic Chromogenic Assay kits contain co-lyophilized lysate/substrate and matched control standard endotoxin (Cat. No. 50-650U also contains LAL Reagent Water).

Bulk kit configurations are also available for those kits. Kinetic Chromogenic LAL and matched control standard endotoxin are packaged separately but should be ordered together. These bulk configurations are made to order, and therefore require a lead time.

Please contact Customer Service for more information.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/kqcl

2° C to 8°C

Benefits

- Sensitivity range from 0.005 to 50 EU/mL
- Less sensitive to product inhibition than assays requiring gel formation
- Available in 192-, 2040-, and 2400-test kit and bulk configurations

Ordering Information – Kinetic-QCL™ Kinetic Chromogenic LAL Assay

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
50-650U	50-650U	Kinetic-QCL™ Kinetic Chromogenic LAL Assay	8 × 24 tests/vial Lysate, 2 vials endotoxin, 3 × 30 mL vial LAL Reagent Water	192 tests	0.005 to 50
50-650NV	50-650NV	Kinetic-QCL™ Kinetic Chromogenic LAL Assay	85 × 24 tests/vial Lysate, 15 vials endotoxin	2,040 tests	0.005 to 50
50-650H	50-650H	Kinetic-QCL™ Kinetic Chromogenic LAL Assay	100 × 24 tests/vial Lysate, 10 vials endotoxin	2,400 tests	0.005 to 50
K50-643L	K50-643L	Kinetic-QCL™ Bulk Kinetic Chromogenic LAL Assay	25 × 24 tests/vial Lysate	600 tests	0.005 to 50
K50-643U	K50-643U	Kinetic-QCL™ Bulk Kinetic Chromogenic LAL Assay	100 × 24 tests/vial Lysate	2,400 tests	0.005 to 50

Control Standard Endotoxin for Kinetic-QCL™ Bulk Kinetic Chromogenic LAL

The Control Standard Endotoxin, derived from *E. coli* 055:B5, is referenced against the USP Reference Standard Endotoxin.

Ordering Information – Control Standard Endotoxin for Kinetic-QCL™ Bulk Kinetic Chromogenic LAL

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
E50-643L	E50-643L	Control Standard Endotoxin for Kinetic-QCL™ Bulk Kinetic Chromogenic LAL, *E. coli* Strain 055:B5	50 EU/mL	25 vials

Related Products	Page
WinKQCL™ Endotoxin Detection and Analysis Software	367
ELx808™ Incubating Absorbance Plate Reader	364
LAL Reagent Grade Multi-well Plates	371
LAL Reagent Water (LRW)	373
Pyrogen-free Test Tubes	370
Tips and Reservoirs	372

QCL-1000™ Endpoint Chromogenic LAL Assay

The QCL-1000™ Endpoint Chromogenic LAL Assay is the most rapid of the LAL tests. This chromogenic LAL method is based on the formation of a yellow color and is measured photometrically at 405–410 nm. With the QCL-1000™ Assay, a multichannel pipette, a dry heat block and a 96-well plate, you can run 96 reaction wells at one time. This assay can also be run in tubes.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/qcl1000

2°C to 8°C

Benefits

- Less sensitive to product inhibition than assays requiring gel formation
- Sensitivity from 0.1 to 1 EU/mL
- Quantitative results in 16 minutes
- Flexible format - use test tubes or 96-well plates
- Can be run with a simple spectrophotometer, no need for incubating absorbance plate reader

Ordering Information – QCL-1000™ Endpoint Chromogenic LAL Assay

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
50-647U	50-647U	QCL-1000™ Endpoint Chromogenic LAL Assay	5 × 24 tests/vial Lysate, 1 × 1 mL vial endotoxin, 2 × 6.5 mL vial chromogenic substrate, 2 × 30 mL vial LAL Reagent Water	120 tests	0.1 to 1
50-648U	50-648U	QCL-1000™ Endpoint Chromogenic LAL Assay	5 × 60 tests/vial Lysate, 2 × 1 mL vial endotoxin, 5 × 6.5 mL vial chromogenic substrate	300 tests	0.1 to 1

Ordering Information – Control Standard Endotoxin for QCL-1000™ Endpoint Chromogenic LAL

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
E50-640	E50-640	Control Standard Endotoxin for QCL-1000™ Endpoint Chromogenic LAL, *E. coli* Strain 0111:B4	Endotoxin, 15 to 40 EU/mL	1 vial	n/a

Related Products	Page
LAL Reagent Water (LRW)	373
Pyrogen-free Test Tubes	370
LAL Reagent Grade Multi-well Plates	371
LAL Reagent Reservoirs	372
Eppendorf® 2–200 µL Biopur® Pipette tips	372
Eppendorf® 50–1000 µL Biopur® Pipette tips	372
Heat Block and Adaptor	373

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Kinetic Turbidimetric LAL Assay Overview

The PYROGENT™-5000 Assay is a quantitative, kinetic assay for the detection of Gram-negative bacterial endotoxin. A sample is mixed with the reconstituted LAL reagent in a 96-well plate and placed in an incubating absorbance plate reader that measures absorbance at 340 nm. The reaction is automatically monitored over time for the appearance of turbidity.

In the presence of endotoxin the lysate will begin to gel, causing the solution to become cloudy or turbid. The time required for the change is inversely proportional to the amount of endotoxin present. The concentration in unknown samples can be calculated from a standard curve.

The PYROGENT™-5000 Assay is perfect for laboratories needing to process a large number of samples. It is ideal for water samples, large volume parenterals, and water rinse from medical devices.

Using our extensive experience and technical expertise with endotoxin detection and its regulatory requirements, Lonza has developed an integrated system to support quantitative endotoxin detection. Each system component has been validated and can be verified. This all leads to reliable, reproducible, and accurate quantitative results.

Each quantitative system incorporates three elements:
- PYROGENT™-5000 Kinetic Turbidimetric LAL Assay
- WinKQCL™ Endotoxin Detection and Analysis Software
- Incubating Absorbance Plate Reader

These elements integrate seamlessly to meet your testing requirements, providing meaningful results that allow you to be confident in your critical decision-making.

Benefits
- Sensitivity range from 0.01 to 100 EU/mL
- Select from a wide range of kit sizes

Applications
- Cost-effective method for water and large volume parenterals

Requirements
- Incubating Absorbance Plate Reader
- WinKQCL™ Software
- LAL Reagent Water
- Pyrogen-free Test Tubes
- LAL Reagent Grade Multi-well Plates

PYROGENT™-5000 Kinetic Turbidimetric LAL Assay

PYROGENT™-5000 kits contain turbidimetric lysate, reconstitution buffer for the lysate, and matched control standard endotoxin. Bulk kit configurations are available with the three assay components packaged separately. The kinetic turbidimetric LAL, reconstitution buffer, and matched Control Standard Endotoxin should be ordered together. These bulk configurations are made to order and therefore require a lead time. Please contact Customer Service for more information.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/turb

2°C to 8°C

Benefits

- Sensitivity range from 0.01 to 100 EU/mL
- Available in 100-, 200-, 2,250-, and 4,500-test kit and bulk configurations

Ordering Information – PYROGENT™-5000 Kinetic Turbidimetric LAL Assay

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
N383	N383	PYROGENT™-5000 Kinetic Turbidimetric LAL Assay	2 × 50 tests/vial Lysate, 2 vials reconstitution buffer, 1 vial endotoxin	100 tests	0.01 to 100
N384	N384	PYROGENT™-5000 Kinetic Turbidimetric LAL Assay	2 × 100 tests/vial Lysate, 2 vials reconstitution buffer, 1 vial endotoxin	200 tests	0.01 to 100
N588	N588	PYROGENT™-5000 Kinetic Turbidimetric LAL Assay	45 × 50 tests/vial Lysate, 45 vials reconstitution buffer, 10 vials endotoxin	2,250 tests	0.01 to 100
N688	N688	PYROGENT™-5000 Kinetic Turbidimetric LAL Assay	45 × 100 tests/vial Lysate, 45 vials reconstitution buffer, 10 vials endotoxin	4,500 tests	0.01 to 100
T50-300L	T50-300L	PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL Assay	25 × 50 tests/vial Lysate	1,250 tests	0.01 to 100
T50-300U	T50-300U	PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL Assay	100 × 50 tests/vial Lysate	5,000 tests	0.01 to 100
T50-600L	T50-600L	PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL Assay	25 × 100 tests/vial Lysate	2,500 tests	0.01 to 100
T50-600U	T50-600U	PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL Assay	100 × 100 tests/vial Lysate	10,000 tests	0.01 to 100

Reconstitution Buffer for PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL

The reconstitution buffer is provided for rehydration of the PYROGENT™-5000 LAL Reagent.

Ordering Information – PYROGENT™-5000 Bulk Kinetic Turbidimetric Reconstitution Buffer

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
B50-300L	B50-300L	PYROGENT™-5000 Bulk Kinetic Turbidimetric Reconstitution Buffer	Reconstitution buffer for T50-300L	25 vials
B50-300U	B50-300U	PYROGENT™-5000 Bulk Kinetic Turbidimetric Reconstitution Buffer	Reconstitution buffer for T50-300U	100 vials
B50-600L	B50-600L	PYROGENT™-5000 Bulk Kinetic Turbidimetric Reconstitution Buffer	Reconstitution buffer for T50-600L	25 vials
B50-600U	B50-600U	PYROGENT™-5000 Bulk Kinetic Turbidimetric Reconstitution Buffer	Reconstitution buffer for T50-600U	100 vials

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Control Standard Endotoxin for PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL

The Control Standard Endotoxin, derived from *E. coli* 055:B5, is referenced against the USP Reference Standard Endotoxin.

Ordering Information – Control Standard Endotoxin for PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity
7460L	7460L	Control Standard Endotoxin for PYROGENT™-5000 Bulk Kinetic Turbidimetric LAL, *E. coli* Strain 055:B5	100 EU/mL	25 vials	n/a

Related Products	Page
WinKQCL™ Endotoxin Detection and Analysis Software	367
ELx808™ Incubating Absorbance Plate Reader	364
LAL Reagent Grade Multi-well Plates	371
LAL Reagent Water (LRW)	373
Pyrogen-free Test Tubes	370

PyroGene™ Recombinant Factor C Assay

The PyroGene™ Recombinant Factor C Assay is an animal-free alternative to LAL that has been accepted by the FDA as an alternative method.* It is based on a recombinantly produced form of Factor C (rFC), the first component in the horseshoe crab clotting cascade. It is activated by endotoxin binding. The active moiety created then acts to cleave a synthetic substrate, which results in the release of a fluorophore. The reaction is run in a 96-well microplate and measured at time zero and after a one-hour incubation in a fluorescence microplate reader using excitation/emission wavelengths of 380/440 nm.

A global, multi-center study demonstrated that the recovery of endotoxin from water and other tested products using the PyroGene™ Recombinant Factor C Assay was comparable to that of LAL-based methods. The results of the assay validation were published in the Pharmacopeial Forum Vol. 36(1) (Jan. – Feb. 2010).

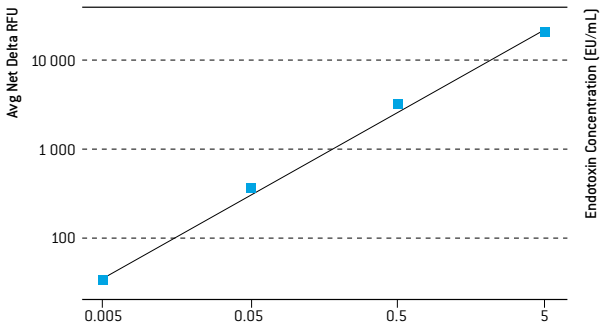
In June 2012, the FDA issued the document "Guidance for Industry - Pyrogen and Endotoxins Testing: Questions and Answers" which allows for the use of a recombinant Factor C based assay as an alternative to Limulus Amebocyte Lysate (LAL)-based assays. In July 2015, rFC became officially recognized by the European Pharmacopoeia (Ph. Eur.) as an alternative endotoxin detection methodology to the LAL and Rabbit Pyrogen Tests in the new draft of Chapter 5.1.10.

USP 28–NF 33 General Notices allows alternative methods if they provide advantages regarding accuracy, sensitivity, precision, selectivity, or adaptability to automation. However, to use these alternative methods for final release testing, one may need to validate the test method on their products as described in the general chapter "Validation of Compendial Procedures" <1225> and it must be shown to give equivalent or better results.

Each quantitative system incorporates three elements:
- PyroGene™ Recombinant Factor C Assay
- WinKQCL™ Endotoxin Detection and Analysis Software
- Fluorescence Plate Reader

These elements integrate seamlessly to meet your testing requirements, providing meaningful results that allow you to be confident in your critical decision-making.

Applications
- Water testing
- In-process testing
- Final release testing
- Testing plant-based material



Standard curve illustrating assay range from 0.005 to 5 EU/mL

Benefits
- Sensitivity range from 0.005 to 5 EU/mL
- Higher endotoxin specificity
- Elimination of false positive glucan reactions
- Less lot-to-lot variability
- Animal-free
- Security of supply
- FDA acknowledged alternative to LAL

Requirements
- Incubating fluorescence feader
- WinKQCL™ Software
- Pyrogen-free test tubes
- LAL reagent grade multi-well plates
- LAL Reagent Water (for larger kits)

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/pyrogene

2°C to 8°C

* According to the FDA "Guidance for Industry - Pyrogen and Endotoxins Testing: Questions and Answers" document from June 2012, alternative assays should be validated as described in the USP General Chapter <1225>, "Validation of Compendial Procedures".

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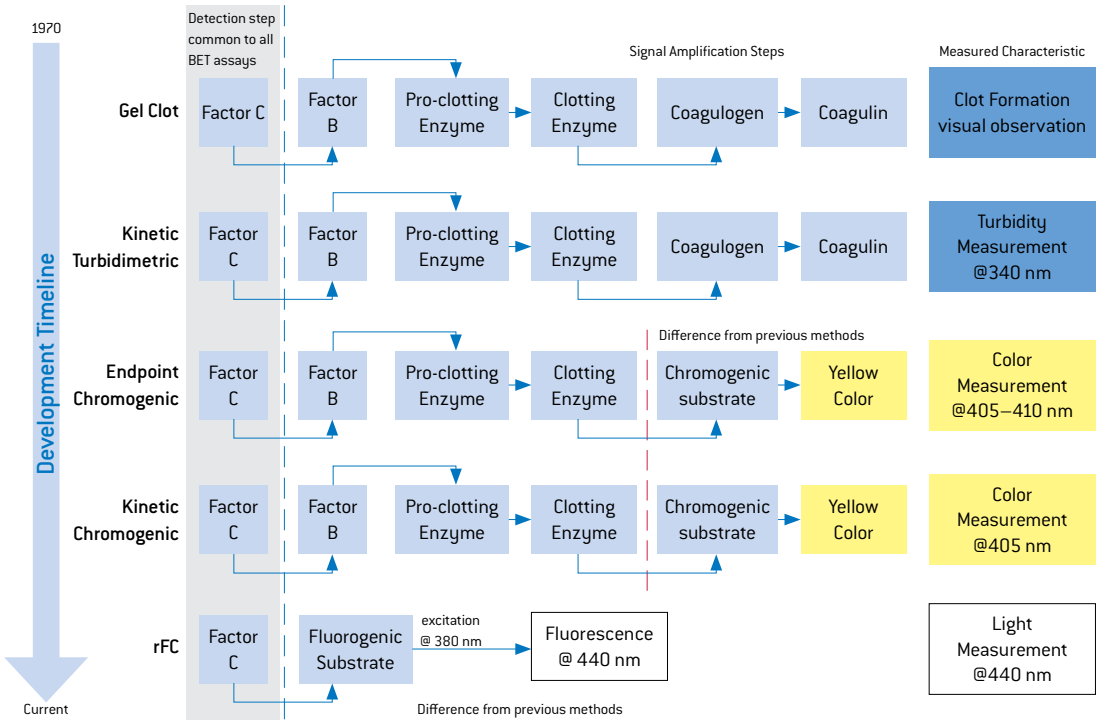
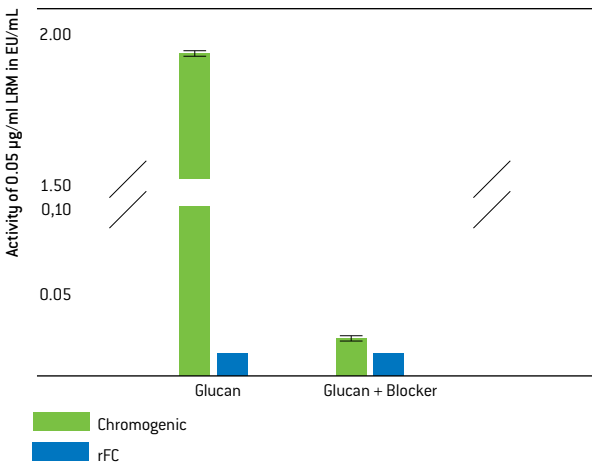
PyroGene™ Recombinant Factor C Assay

Continued

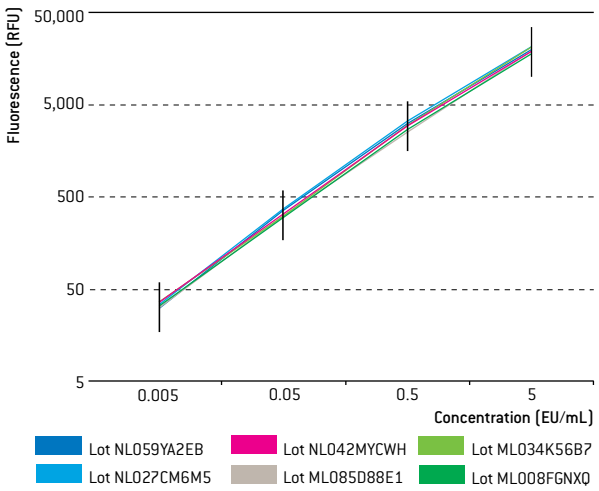
Comparison of Amplification Methods in Bacterial Endotoxin Detection Assays



rFC is the same binding protein operating in the LAL assay. The activated recombinant Factor C enzyme cleaves a substrate directly instead of activating another enzyme in a series (the LAL cascade). The substrate has a fluorescent tag, which gives a wide dynamic range with better resolution.



Comparison of reactivity towards glucans between kinetic chromogenic LAL and rFC. The false positive signal from the LAL assay is reduced in the presence of a glucan blocker. rFC does not detect any glucan activity; it is endotoxin-specific.



Endotoxin standard curves using 6 different lots of rFC. The log net fluorescence is proportional to the log endotoxin concentration and is linear in the 0.005 - 5 EU/mL range. Lot-to-lot standard curves exhibit excellent reproducibility.

PyroGene™ Validation Timeline

A possible validation scheme is outlined below. One validation can be accomplished in as little as 5 days, assuming that the product has been previously validated with a quantitative LAL method. The validation scheme is identical to that which would be needed for any LAL-based method with just the addition of one extra step– "Validation of alternative method". Lonza offers a full validation protocol that can be followed for your convenience. For further information, please contact Scientific Support or your local sales representative.

Preparation	Initial Qualification	Inhibition/ Enhancement Testing	Validation of Alternative Method	Product Validation
– IOPQ – Sensitivity assay for rFC – Initial qualification of current and rFC method and analyst	– Not necessary if previously qualified reagent lots are used	– Only for rFC – Time depending on extent of testing required to find a suitable dilution/pre-treatment	– Tests with both current and rFC method – Good planning can save time	– 3 production lots
2 days	0.5 days	>0.5 days (minimum)	>1.5 days (minimum)	0.5 days

Ordering Information –PyroGene™ Recombinant Factor C Endpoint Fluorescent Assay

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
50-658U	**50-658U**	PyroGene™ Recombinant Factor C Endpoint Fluorescent Assay	2 × 96 tests/vial rFC enzyme solution, 2 × 6 mL vial fluorogenic substrate, 2 × 5 mL vial rFC assay buffer, 2 vials endotoxin, 2 × 30 mL vial LAL Reagent Water	192 tests	0.005 to 5
50-658NV	**50-658NV**	PyroGene™ Recombinant Factor C Endpoint Fluorescent Assay	30 × 96 tests/vial rFC enzyme solution, 30 × 6 mL/vial fluorogenic substrate, 30 × 5 mL/vial rFC assay buffer, 10 vials endotoxin	2,880 tests	0.005 to 5

Related Products	Page
Pyrogen-free Test Tubes	370
LAL Reagent Grade Multi-well Plates	371
LAL Reagent Reservoirs	372
Eppendorf® 2–200 µL Biopur® Pipette tips	372
Eppendorf® 50–1000 µL Biopur® Pipette tips	372
PyroWave XM™ Fluorescence Plate Reader	365

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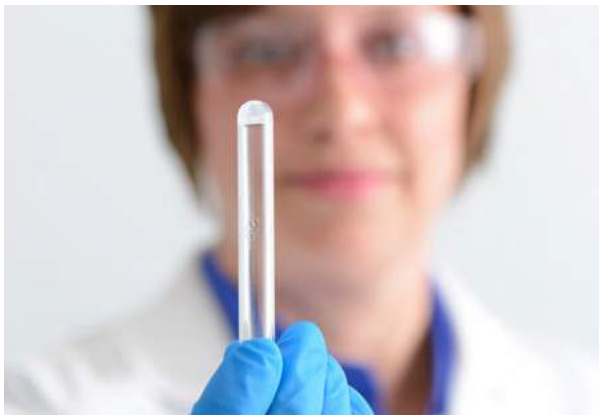
PYROGENT™ Gel Clot LAL Assay Overview

The PYROGENT™ Gel Clot LAL Assay is a qualitative LAL test for Gram-negative bacterial endotoxin. The gel clot assay is performed in tubes that are placed in a water bath or dry heat block at 37°C. After a one-hour incubation period, the tubes are inverted 180°. A firm clot that stays in the bottom of the tube indicates a positive reaction. If liquid flows down the side of the tube, the result is negative for endotoxin.

Like other enzymatic reactions, the LAL assay is pH dependent. The PYROGENT™ lysate formulation contains a buffer to help with these adjustments. As a result, many products will not require pH adjustments prior to testing.

PYROGENT™ Gel Clot LAL kits are available in two formats:

	Lysate	Matched Endotoxin
PYROGENT™ Gel Clot LAL	■	
PYROGENT™ Plus Gel Clot LAL	■	■



Benefits

- Easy-to-read qualitative results
- Simple LAL test not requiring sophisticated instrumentation and software
- Select from a wide range of kit sizes and sensitivities

Applications

- Water testing
- In-process testing
- Final release testing
- Testing plant-based material
- Testing acidic/basic material

Requirements

- A water bath or dry heat block
- LAL Reagent Water (LRW)
- Pyrogen-free test tubes

PYROGENT™ Gel Clot LAL Assay

PYROGENT™ Gel Clot LAL Assay standard kit sizes include 250 tests and 80 tests. Both the 250- and 80-test kits require depyrogenated 10 × 75 mm glass reaction tubes to run the assay.

In addition, kits of PYROGENT™ Gel Clot LAL Single Test Vials are available, which consist of 25 single use vials of lysate. Each single test vial serves as one reaction tube for an assay.

These kits do not include a matched control standard endotoxin. However, the standard can be purchased separately (Control Standard Endotoxin, page 361).

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/gelclot

2°C to 8°C

Benefits

- Sensitivities of 0.03, 0.06, 0.125, and 0.25 EU/mL available
- Easy-to-read qualitative results
- Also available as single test vials and as bulk kits

Ordering Information – PYROGENT™ Gel Clot LAL Assay (without endotoxin)

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
N183-06	N183-06	PYROGENT™ Gel Clot LAL Assay (without endotoxin)	5 × 16 tests/vial Lysate	80 tests	0.06
N183-125	N183-125	PYROGENT™ Gel Clot LAL Assay (without endotoxin)	5 × 16 tests/vial Lysate	80 tests	0.125
N194-03	N194-03	PYROGENT™ Gel Clot LAL Assay (without endotoxin)	5 × 50 tests/vial Lysate	250 tests	0.03
N194-06	N194-06	PYROGENT™ Gel Clot LAL Assay (without endotoxin)	5 × 50 tests/vial Lysate	250 tests	0.06
N194-125	N194-125	PYROGENT™ Gel Clot LAL Assay (without endotoxin)	5 × 50 tests/vial Lysate	250 tests	0.125
N184-25	N184-25	PYROGENT™ Gel Clot LAL Assay (without endotoxin)	5 × 50 tests/vial Lysate	250 tests	0.25
N189-06	N189-06	PYROGENT™ Gel Clot LAL Assay (without endotoxin) Single Test Vials	25 single tests/vial Lysate	25 vials	0.06
N189-125	N189-125	PYROGENT™ Gel Clot LAL Assay (without endotoxin) Single Test Vials	25 single tests/vial Lysate	25 vials	0.125
N189-25	N189-25	PYROGENT™ Gel Clot LAL Assay (without endotoxin) Single Test Vials	25 single tests/vial Lysate	25 vials	0.25

Related Products	Page
Control Standard Endotoxin for Gel Clot LAL	361
Bulk kits	360
LAL Reagent Water (LRW)	373
Pyrogen-free Test Tubes	370
Eppendorf® 2–200 µL Biopur® Pipette tips	372
Eppendorf® 50–1000 µL Biopur® Pipette tips	372

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PYROGENT™ Plus Gel Clot LAL Assay

The PYROGENT™ Plus Gel Clot LAL Assay combines PYROGENT™ LAL with a matched control standard endotoxin together in one kit box. Standard kit sizes include 4,000 tests, 200 tests, or 64 tests. The 4,000-, 200-, and 64-test kits require depyrogenated 10 × 75 mm glass reaction tubes to run the assay. In addition, kits of PYROGENT™ Plus Gel Clot LAL Single Test Vials are available that include 24 single-use test vials of lysate serving as the reaction tubes.

For your convenience, the Certificate of Analysis documenting the FDA and USP required RSE/CSE correlation is available online:

www.lonza.com/coa

www.lonza.com/gelclot

Benefits

- Sensitivities of 0.03, 0.06, 0.125 and 0.25 EU/mL available
- No need to purchase CSE separately
- Also available as single test vials and as bulk kits

2°C to 8°C

Ordering Information – PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin)

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
N283-06	N283-06	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin)	4 × 16 tests/vial Lysate, 1 vial endotoxin	64 tests	0.06
N283-125	N283-125	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin)	4 × 16 tests/vial Lysate, 1 vial endotoxin	64 tests	0.125
N294-03	N294-03	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin)	4 × 50 tests/vial Lysate, 1 vial endotoxin	200 tests	0.03
N294-06	N294-06	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin)	4 × 50 tests/vial Lysate, 1 vial endotoxin	200 tests	0.06
N294-125	N294-125	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin)	4 × 50 tests/vial Lysate, 1 vial endotoxin	200 tests	0.125
N284-25	N284-25	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin)	4 × 50 tests/vial Lysate, 1 vial endotoxin	200 tests	0.25
N494-03	N494-03	PYROGENT™ Plus Bulk Gel Clot LAL Assay (with endotoxin)	80 × 50 tests/vial Lysate, 20 vials endotoxin	4,000 tests	0.03
N494-06	N494-06	PYROGENT™ Plus Bulk Gel Clot LAL Assay (with endotoxin)	80 × 50 tests/vial Lysate, 20 vials endotoxin	4,000 tests	0.06
N494-125	N494-125	PYROGENT™ Plus Bulk Gel Clot LAL Assay (with endotoxin)	80 × 50 tests/vial Lysate, 20 vials endotoxin	4,000 tests	0.125
N288-25	N288-25	PYROGENT™ Plus Bulk Gel Clot LAL Assay (with endotoxin)	80 × 50 tests/vial Lysate, 20 vials endotoxin	4,000 tests	0.25
N289-06	N289-06	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin), Single Test Vials	24 single tests/vial Lysate, 1 × 1 mL vial endotoxin	24 tests	0.06
N289-125	N289-125	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin), Single Test Vials	24 single tests/vial Lysate, 1 × 1 mL vial endotoxin,	24 tests	0.125
N289-25	N289-25	PYROGENT™ Plus Gel Clot LAL Assay (with endotoxin), Single Test Vials	24 single tests/vial Lysate, 1 × 1 mL vial endotoxin	24 tests	0.25

Related Products	Page
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Pyrogen-free Test Tubes	370
Eppendorf® 2–200 µL Biopur® Pipette tips	372
Eppendorf® 50–1000 µL Biopur® Pipette tips	372

PYROGENT™ Bulk Gel Clot LAL Assay

Bulk kit configurations of PYROGENT™ Gel Clot LAL are available for laboratories using large volumes of reagents. These configurations are made to order and production lead times are required. Please inquire with your sales representative for more information.

Benefits

- Bulk configurations for large volume use
- Bulk kits with and without endotoxin standard available
- Sensitivities of 0.03, 0.06, 0.125, and 0.25 EU/mL available

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/gelclot

2°C to 8°C

Ordering Information – PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
E194L-03	E194L-03	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	25 × 50 tests/vial Lysate	1,250 tests	0.03
E194L-06	E194L-06	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	25 × 50 tests/vial Lysate	1,250 tests	0.06
E194L-125	E194L-125	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	25 × 50 tests/vial Lysate	1,250 tests	0.125
E209L-25	E209L-25	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	25 × 50 tests/vial Lysate	1,250 tests	0.25
F245U-06	F245U-06	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	100 × 16 tests/vial Lysate	1,600 tests	0.06
F245U-125	F245U-125	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	100 × 16 tests/vial Lysate	1,600 tests	0.125
E194U-03	E194U-03	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	100 × 50 tests/vial Lysate	5,000 tests	0.03
E194U-06	E194U-06	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	100 × 50 tests/vial Lysate	5,000 tests	0.06
E194U-125	E194U-125	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	100 × 50 tests/vial Lysate	5,000 tests	0.125
E209U-25	E209U-25	PYROGENT™ Bulk Gel Clot LAL Assay (without endotoxin)	100 × 50 tests/vial Lysate	5,000 tests	0.25

Related Products	Page
LAL Reagent Water (LRW)	373
Pyrogen-free Test Tubes	370
Eppendorf® 2–200 µL Biopur® Pipette tips	372
Eppendorf® 50–1000 µL Biopur® Pipette tips	372
Control Standard Endotoxin for PYROGENT™ Gel Clot LAL	361

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Control Standard Endotoxin for PYROGENT™ Gel Clot LAL

Lonza's Control Standard Endotoxin is referenced against the USP Reference Standard Endotoxin.

Certificates of Analysis showing potency are available online:
www.lonza.com/coa

2°C to 8°C

Ordering Information – Control Standard Endotoxin for PYROGENT™ Gel Clot LAL

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size	Sensitivity (EU/mL)
N186	N186	Control Standard Endotoxin for PYROGENT™ Gel Clot LAL Assays	Endotoxin, *E. coli* 055:B5	5 vials	n/a
7360L	7360L	Bulk Control Standard Endotoxin for PYROGENT™ Gel Clot LAL Assays	Endotoxin, *E. coli* 055:B5	25 vials	n/a

Related Products	Page
Gel Clot LAL Assays	358
LAL Reagent Water (LRW)	373
Pyrogen-free Test Tubes	370
Eppendorf® 2–200 µL Biopur® Pipette tips	372
Eppendorf® 50–1000 µL Biopur® Pipette tips	372

QC Insider™ Toolbox

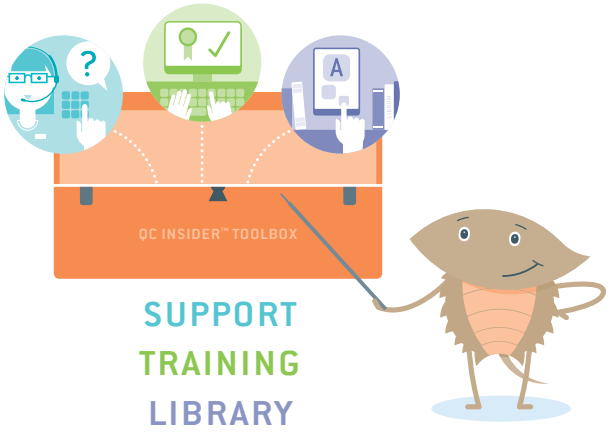
Endotoxin Expertise At Your Fingertips™

The QC Insider™ Toolbox has been designed for endotoxin testing novices as well as experts to provide endotoxin testing expertise at any level. The online portal contains a comprehensive offering of beginner and advanced support tools, a wide range of training resources, and a library of information that can be accessed at any time and from anywhere with internet access. The QC Insider™ Toolbox is organized into three categories so that users can easily navigate directly to the support tool they need.

The QC Insider™ Support offers one-on-one guidance, detailed information about software support, recertification and testing services, reader installation and maintenance, and workflow optimization.

The QC Insider™ Training contains self-directed training resources that will help users increase their endotoxin testing expertise, including a series of how-to videos that demonstrate different assay procedures.

The QC Insider™ Library consists of technical resources such as package inserts, quick guides, and technical tips that will help lead to success with endotoxin testing.



Become a QC Insider™ Expert today and ensure the support you need is always within reach.

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Instrumentation and Software

Instrumentation and Software

ELx808™ Incubating Absorbance Plate Reader	364
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WinKQCL™ Endotoxin Detection and Analysis Software	367

ELx808™ Incubating Absorbance Plate Reader

For Kinetic-QCL™ and PYROGENT™-5000 Kinetic LAL Assays

The ELx808™ Incubating Absorbance Plate Reader has been validated as part of our quantitative endotoxin detection systems. This 96-well microplate reader features 4 insulated incubating zones, providing well-to-well temperature uniformity available on the market. Utilizing interference filters instead of monochromators, the reader is optimized for the LAL-based endotoxin tests and comes pre-configured with the following interference filters: 340nm, 405nm, 450nm, 490nm, and 630nm. The ELx808™ Reader seamlessly interfaces with Lonza's WinKQCL™ Endotoxin Detection and Analysis Software.

For the kinetic and endpoint LAL assays, the ELx808™ Reader is highly reliable and easy to use. It features fully-automated kinetic reads of the 96-well plate via the WinKQCL™ Software. This reader can also be used as a standard spectrophotometer and results can be read from the QCL-1000™ Endpoint Chromogenic LAL Assay and ELISA Assays.

On-site service and preventive maintenance contracts are available to help ensure that your instrument is working properly.

Benefits

- Interference filters provide up to 5x the light energy of monochromator-based filters
- Excellent temperature uniformity
- Precise and accurate
- Cost effective filter-based reader

ELx808™ Absorbance Reader Specifications	
Wavelength Range	340 to 900 nm
Filters Supplied	340, 405, 450, 490 and 630 nm
Absorbance Range	0.000 to 4.000 OD @ 400 to 900 nm 0.000 to 3.000 OD @ 340 to 400 nm
Temperature Control	4°C above ambient to 50°C
Read Method	Kinetic or endpoint under WinKQCL™ Control
Light Source	Tungsten halogen bulb
Dimensions	16-inches deep × 15.5-inches wide × 8.75-inches high (40.6 cm × 39.4 cm × 22.2 cm)
Weight	35 lb (15.9 kg)

Ordering Information – ELx808™ Reader

Cat. No. NA	Cat. No. EU	Product Name	Product Description
25-315S	25-315S	ELx808™ Reader	Incubating 8-channel absorbance plate reader
25-342	25-342	Stepped Neutral Density Plate*	For optical density validation across eight channels
7260522	7260522	BioTek™ Absorbance Test Plate	For alignment, repeatability, and accuracy validation
05105	LAL3400508	Replacement Bulb for ELx808™ Reader	
196171	75053	Computer Connection Cable for ELx808™ Reader	9 pin female to 25 pin female RS232 cable
196004		UPS-APC 1500VA	Uninterruptible power supply with LCD, 120 V output (US edition)
	BE00196004	UPS-APC 1500VA	Uninterruptible power supply with LCD, 230 V output (European edition)
196005	196005	4-Port Serial PCI Card	Standard/low profile PCI card
25-361	25-361	USB to Serial Port Converter	10 cm cable with LED communication indicator lights

*Recertification service is available (see Recertification Services. page 390)

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PyroWave™ XM Fluorescence Reader

For the PyroGene™ rFC Endpoint Fluorescent Assay

The PyroWave™ XM Fluorescence Reader is a part of the quantitative endotoxin detection system that supports the PyroGene™ rFC Endpoint Fluorescent Assay. This reader replaces the Lonza FLx800™ LBS reader, bringing a new generation in fluorescence technology to users of the PyroGene™ Assay. Optimized specifically for Lonza's PyroGene™ Assay, this reader brings new and improved technology to the laboratory. The PyroWave™ XM Reader offers numerous enhancements in incubation, optics, automation compatibility, and overall reader maintenance and robustness. Controlled by Lonza's WinKQCL™ Endotoxin Detection and Analysis Software, version 5.2 and higher, Lonza delivers a high performance and easy to use system for users interested in an alternative to the horseshoe crab-based endotoxin detection test methods.

On-site service and preventive maintenance contracts are available to help ensure that your instrument is working properly.

Benefits

- Improved PyroGene™ Assay performance
- Reduced reader maintenance
- Automation compatible
- High performance optics
- 4-Zone™ Incubation at 37°C

PyroWave™ XM Fluorescence Reader Specifications	
Read capabilities	Fluorescence, luminescence**, TRF**, FP**
Read position	Top read
Light source	Xenon flash lamp
Detection	High performance photo multi-plier tube
Fluorescence sensitivity	Fluorescein @ 1 pM/well in a 96-well plate
Wavelength range	200 to 850 nm**
Filters	One easy-to-swap filter cube with the following filter configuration:

Assay Type	Excitation Filter (nm)	Emission Filter (nm)	Dichroic Mirror (nm)
PyroGene™	380/20	440/30	400
Fluorescein	485/20	528/20	510

**Additional filter cubes and filters must be purchased from reader manufacturer for additional wavelengths and read capabilities

Temperature control	±0.2 °C at 37 °C
Power	100 – 240 Volts AC 50/60 Hz
Dimensions	39.1 cm W x 47.2 cm D x 32.8 cm H (15.4" W x 18.6" D x 12.9" H)
Weight	22.5 kg (50 lbs)

Ordering Information – PyroWave™ XM Reader

Cat. No. NA	Cat. No. EU	Product Name	Product Description
25-345S	25-345S	PyroWave™ XM Fluorescence Reader	Incubating fluorescence reader
196004		UPS-APC 1500VA	Uninterruptible power supply with LCD, 120 V output (US edition)
	BE00196004	UPS-APC 1500VA	Uninterruptible power supply with LCD, 230 V output (European edition)
196005	196005	4-Port Serial PCI Card	Standard/low profile PCI card
204511	7160013	Sodium Fluorescein Liquid Test Kit	For reader operational qualification and PM verification

Related Products	Page
WinKQCL™ Endotoxin Detection and Analysis Software	367
PyroGene™ rFC Endpoint Fluorescent Assay	354

PyroTec™ Liquid Handling System

The PyroTec™ Liquid Handling System is a robotic workstation to help automate endotoxin detection testing. The system includes a user-defined platform size to accommodate tips, reagent troughs and 96-well plates. The robotic arm picks up tips and dispenses samples and reagents to 96-well plates. Heating units can incubate plates prior to delivery into plate readers.

WinKQCL™ Software is fully integrated with the Tecan® Freedom EVOware® Software, allowing assay templates to be executed using robotic scripts for the PyroTec™ Liquid Handling System.

The PyroTec™ System can be tailored to your testing needs. Contact your local Lonza sales representative or scientific support for additional information.

On-site service and preventive maintenance contracts are available to help ensure that your instrument is working properly.

Benefits

- Flexible platform to automate filling of assay plates
- Help high throughput labs manage their large daily sample requirements

Ordering Information – PyroTec™ Liquid Handling System

Cat. No. NA	Cat. No. EU	Product Name	Product Description
25-601	25-601	PyroTec™ 200 Liquid Handling System	Robotic workstation for filling nine 96-well plates at one time
25-602	25-602	PyroTec™ 150 Liquid Handling System	Robotic workstation for filling three 96-well plates at one time
25-603	25-603	PyroTec™ 150 Positive ID System	Robotic barcode scanner

Related Products	Page
WinKQCL™ Endotoxin Detection and Analysis Software	367
Kinetic-QCL™ Kinetic Chromogenic LAL Assay	349
PYROGENT™-5000 Kinetic Turbidimetric LAL Assay	352
PyroGene™ rFC Endpoint Fluorescent Assay	354

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WinKQCL™ Endotoxin Detection and Analysis Software

Quantitative methods such as the Kinetic-QCL™ Assay generate significant amounts of raw data that require careful analysis before reporting can take place. The WinKQCL™ Software offers a fully integrated solution for your quantitative endotoxin detection testing, data management, and reporting needs.

WinKQCL™ 5 Software meets 21 CFR Part 11 technical requirements for electronic records, signatures, audit trails and database archiving. Reader validation tests can be run from the WinKQCL™ Software and are stored in the same database. The built-in database backup and maintenance scheduler makes it easy to maintain the system.

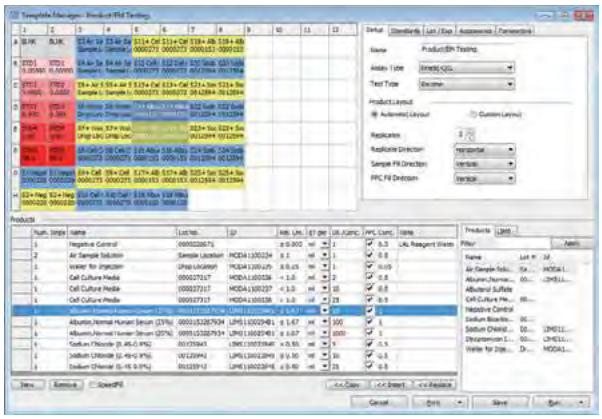
Benefits

- Kinetic SmartStop™ monitoring feature to address split pair and other reaction conditions, without sacrificing time waiting for a fixed number of reads
- Enterprise level IT features including wide area network support, ability to work across time zones, application virtualization, Active Directory® integration, and data segregation by lab
- Bi-directional interface with 3rd party database systems
- Customizable endotoxin test reports
- Multi-language user interface: English, French, German, Italian, Japanese, Spanish, Portuguese, Simplified Chinese and Traditional Chinese

Applications

- For use with the PyroWave™ XM, ELx808™ , and FLx800™ Readers. Extended reader integration now includes Molecular Devices® SpectraMax®, Gemini™ and VersaMax™ Readers; and the BioTek® Eon™ and Synergy™ 2 Readers. The software is also interfaced with the Tecan® Sunrise™ Reader
- Tecan® EVOware® interface integration for PyroTec™ Liquid Handling System
- Supports all quantitative endotoxin detection assays from Lonza including QCL-1000™ Endpoint Chromogenic LAL, Kinetic-QCL™ Kinetic Chromogenic LAL, PYROGENT™-5000 Kinetic Turbidimetric LAL and PyroGene™ rFC Endpoint Fluorescent Assays
- Installation as a simple standalone system or as an interface with multiple robots and readers in multiple labs around the world, all storing data in a single database

The user-friendly and flexible Template Manager allows you to customize plate layout with a click of a mouse using the SpeedFill™ and drag 'n drop features. The interactive and enhanced trending tools provide meaningful results on demand, helping you easily detect drift and enabling you to make proactive decisions.



The endotoxin detection instruments and software from Lonza are available fully supported with Installation, Operational and Performance Qualification (IOPQ) manuals and a WinKQCL™ 5 Software Validation Package. In addition, a trained specialist from Lonza can perform the IOPQ of the complete system to help you with your system validation process. Please inquire with your local sales representative for further details.

WinKQCL™ Endotoxin Detection and Analysis Software

Continued

Ordering Information – WinKQCL™ Endotoxin Detection and Analysis Software

Cat. No. NA	Cat. No. EU	Product Name	Product Description
25-501	**25-501**	WinKQCL™ 5 Software Package	Installation DVD, workgroup license, reader license
25-502	**25-502**	WinKQCL™ 5 Workgroup License	Additional workgroup license
25-503	**25-503**	WinKQCL™ 5 Reader License	Additional reader license
25-504	**25-504**	WinKQCL™ 5 Qualification Manual	DVD containing IOPQ files for software and readers
25-505	**25-505**	WinKQCL™ 5 Validation Package	DVD containing software validation information
25-339S	25-339S	System Qualification Service	IQ/OQ/PQ validation on site, labor only
25-501SUP	25-501SUP	Annual WinKQCL™ Support for Cat. No. 25-501	Advanced IT/software support
25-502SUP	25-502SUP	Annual WinKQCL™ Support for Cat. No. 25-502	Advanced IT/software support
25-503SUP	25-503SUP	Annual WinKQCL™ Support for Cat. No. 25-503	Advanced IT/software support

Ordering Information – WinKQCL™ Software Delivered Immediately by Email

Cat. No. NA	Cat. No. EU	Product Name	Product Description
25-501E	**25-501E**	E-Delivered WinKQCL™ 5 Software Package	Installation files, workgroup license, reader license
25-502E	**25-502E**	E-Delivered WinKQCL™ 5 Workgroup License	Additional workgroup license
25-503E	**25-503E**	E-Delivered WinKQCL™ 5 Reader License	Additional reader license
25-504E	**25-504E**	E-Delivered WinKQCL™ 5 Qualification Manual	IOPQ files for software and readers
25-505E	**25-505E**	E-Delivered WinKQCL™ 5 Validation Package	File containing software validation information

Related Products	Page
Kinetic-QCL™ Kinetic Chromogenic LAL Assay	349
PYROGENT™-5000 Kinetic Turbidimetric LAL Assay	352
PyroGene™ rFC Endpoint Fluorescent Assay	354
ELx808™ Incubating Absorbance Reader	364
PyroWave™ XM Incubating Fluorescence Reader	365

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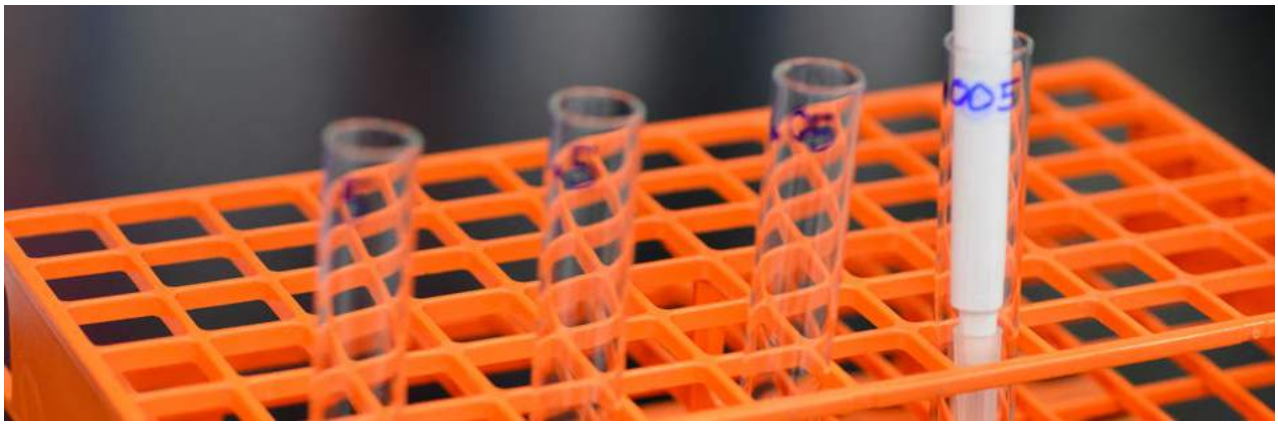
Accessory Products



Accessory Products

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Introduction

In addition to the endotoxin detection kits, instruments, and software, Lonza offers many of the accessory items necessary to run endotoxin detection assays. Many of the items have been tested with the Kinetic-QCL™ Kinetic Chromogenic LAL Assay to help ensure their compatibility with our endotoxin detection methods. We also offer products such as the Endotoxin Challenge Vials™ to help with your oven depyrogenation validations.

Test Tubes

All test tubes are made from USP Type I flint borosilicate glass.

Both productsN201 and N205 are recommended for use as reaction tubes in gel clot assays. N201 are provided with polypropylene screw caps. Product number N207 is recommended for dilution of endotoxin standards and test samples for all endotoxin detection assays.

Benefits

– Certified to contain less than 0.005 EU/mL endotoxin

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/accessories

Ordering Information – Test Tubes

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
N207	N207	Pyrogen-free Test Tubes	Without caps, 13 × 100 mm	30/foil pack
N201	N201	Pyrogen-free Test Tubes	With caps, 10 × 75 mm	50/box
N205	N205	Pyrogen-free Test Tubes	Without caps, 10 × 75 mm	50/foil pack

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Sample Containers

Sample containers are intended for transporting product samples for endotoxin analysis or sample storage. Proper container and storage conditions need to be validated for each individual sample.

Products 80-507L and 80-507U contain 10 mL glass vials with screw caps. Products BE2098 and BE2099 are plastic sample containers that offer greater capacity at a reduced cost.

Benefits

– Certified to contain less than 0.005 EU/mL endotoxin

For your convenience, Certificates of Analysis are available online:

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www.lonza.com/accessories

Ordering Information – Sample Containers

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
80-507L	80-507L	Sample Containers	Depyrogenated, 10 mL glass bottle with screw cap	25/box
80-507U	80-507U	Sample Containers	Depyrogenated, 10 mL glass bottle with screw cap	100/box
	BE2098	Polypropylene Sample Containers	Endotoxin tested, 50 mL tubes	50/pack
	BE2099	Polystyrene Sample Containers	Endotoxin tested, 15 mL tubes	50/pack

Plates

These 96-well plates can be used with the QCL-1000™ Endpoint Chromogenic LAL Assay, Kinetic-QCL™ Kinetic Chromogenic LAL Assay, PYROGENT™-5000 Kinetic Turbidimetric LAL Assay and PyroGene™ rFC Endpoint Fluorescent Assay. Each case contains individually wrapped plates.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

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Benefits

– Certified to contain less than 0.0005 EU/well endotoxin
– Certified for compatibility with the endotoxin detection assays
– Certified to be free from inhibition

Ordering Information – Plates

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
25-340	BE25-340	LAL Reagent Grade Multi-well Plates	96-well plates, endotoxin-tested (<0.0005 EU/well)	50/case

Pipette Tips

Pyrogen-free pipette tips are to be used when testing with any of our endotoxin detection systems. Eppendorf® Biopur® pipette tips are certified to contain <0.001 EU/mL endotoxin. The new design of the tips allows compatibility with different pipettors. Catalog Number 25-416 is for use with multi-channel pipettors. Products BE25-413 and BE25-414 are certified to contain <0.005 EU/mL endotoxin. They can be used with pipettes from different manufacturers. Eppendorf® Combitips® are for use with a multi-step pipette.

Benefits

– Endotoxin tested
– Broad offering for various pipette types

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/accessories

Ordering Information – Pipette Tips

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
25-415	BE25-415	Eppendorf® 2–200 µL Biopur® Pipette Tips	< 0.001 EU/mL	5 trays/pkg; 96 tips/tray
25-416	BE25-416	Eppendorf® 2–300 µL Biopur® Pipette Tips	< 0.001 EU/mL, for multi-channel pipettors	5 trays/pkg; 96 tips/tray
25-417	BE25-417	Eppendorf® 50–1000 µL Biopur® Pipette Tips	< 0.001 EU/mL	5 trays/pkg; 96 tips/tray
	89634	Eppendorf® Combitips®, 0.5 mL	Single packed	100
	89650	Eppendorf® Combitips®, 2.5 mL	Single packed	100
	89669	Eppendorf® Combitips®, 5 mL	Single packed	100
	89677	Eppendorf® Combitips®, 10 mL	Single packed	100
	BE10035	Eppendorf® Pipette Tips, 2–200 µL	Endotoxin tested, single packed	100/box
	BE10051	Eppendorf® Pipette Tips, 50–1000 µL	Endotoxin tested, single packed	100/box
	BE25-413	LAL Reagent Grade Pipette Tips, 2–200 µL	<0.005 EU/mL	10 × 96 tips
	BE25-414	LAL Reagent Grade Pipette Tips, 50–1250 µL	<0.005 EU/mL	10 × 96 tips
	BE7521	Pipette BD Falcon™, 1 mL	Endotoxin tested, single packed	100
	BE7507	Pipette BD Falcon™, 2 mL	Endotoxin tested, single packed	100
	BE7543	Pipette BD Falcon™, 5 mL	Endotoxin tested, single packed	200
	BE7551	Pipette BD Falcon™, 10 mL	Endotoxin tested, single packed	200

Reservoirs

The LAL Reagent Reservoirs are for use with multi-channel pipettors when adding reagents to a 96-well plate. The reservoirs are provided in a zip closure bag enabling you to conveniently store unused reservoirs for later use.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/accessories

Benefits

– Certified to contain less than 0.005 EU/mL endotoxin

Ordering Information – LAL Reagent Reservoirs

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
190035	190035	LAL Reagent Reservoirs	<0.005 EU/mL	10/pack

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Dry Heat Block, Inserts and Vortex Mixer

The dry heat block is used for incubation of LAL gel clot assays. The aluminum block with lid adaptor for a dry heat block allows a 96-well plate to be incubated at 37°C for use with QCL-1000™ Endpoint Chromogenic LAL Assay.

www.lonza.com/accessories

Ordering Information – Dry Heat Block, Inserts and Vortex Mixer

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
25-038A	25-038A	Aluminum Block with Lid	Heat block insert for 96-well plate	Each
	BEF3503	Aluminum Insert Block for Techne Dry Heat Block		For 20 tubes
	BEDB-2D	Techne Dry Heat Block	Digital, from 25°C to 100°C, requires 2 × BEF3503	For 40 tubes
	FDB03DD	Techne Dry Heat Block	Digital, from 25°C to 100°C, requires 3 × BEF3503	For 60 tubes
	BENP5051	Vortex Genie® 2		

LAL Reagent Water

LAL Reagent Water is recommended for reconstituting LAL reagents, as well as for the dilution of control standard endotoxin and test samples for endotoxin testing. LAL Reagent Water is equivalent to Water for Bacterial Endotoxins Test (BET). In addition to USP-required WFI tests, Lonza tests LAL Reagent Water for compatibility with our endotoxin detection assays.

Benefits

- Certified to contain less than 0.005 EU/mL endotoxin
- Available in a variety of sizes
- Certified for Positive Product Control Recovery within 75 to 150%

2°C to 8°C (W50-640)

15°C to 30°C (W50-100, W50-500, W50-1000)

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/accessories

Ordering Information – LAL Reagent Water

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
W50-640	W50-640	LAL Reagent Water	<0.005 EU/mL, 30 mL	1 bottle
W50-100	W50-100	LAL Reagent Water	<0.005 EU/mL, 100 mL	1 bottle
W50-500	W50-500	LAL Reagent Water	<0.005 EU/mL, 500 mL	1 bottle
W50-1000	W50-1000	LAL Reagent Water	<0.005 EU/mL, 1000 mL	1 bottle

β-G-Blocker

β-D-glucans can produce false positive results in LAL assays. Some examples of glucan sources include yeast and cellulosic materials such as hemodialysis filters. Our β-G-Blocker may be used with any of our LAL assays.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/accessories

Benefits

- Certified to contain less than 0.005 EU/mL endotoxin
- Functionality tested to demonstrate a reduction of enhancement caused by β-D-glucans

2°C to 8°C

Ordering Information – β-G-Blocker

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
N190	N190	β-G-Blocker	Glucan blocker, 5 mL/vial	5 vials

PYROSPERSE™ Dispersing Agent

PYROSPERSE™ Dispersing Agent, helps eliminate endotoxin binding or masking in some samples – solving problems of inhibitory behavior. Examples of samples that may show endotoxin binding behavior include plasma protein fractions, electrolyte solutions, and lipid emulsions. PYROSPERSE™ Dispersing Agent may be used with any of our LAL kits.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/accessories

Benefits

- Endotoxin and functionality tested

2°C to 30°C (unopened)

Ordering Information –PYROSPERSE™ Dispersing Agent

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
N188	N188	PYROSPERSE™ Dispersing Agent	5 mL/vial	5 vials

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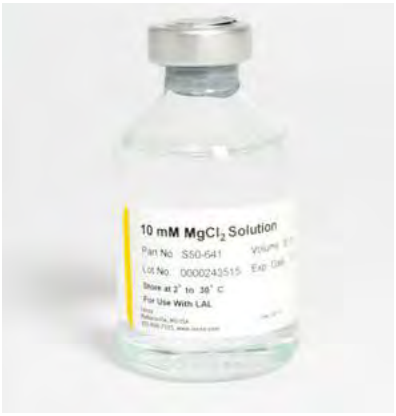
$MgCl_2$

$MgCl_2$ can be used as the sample diluent when attempting to overcome inhibitory chelation effects. Examples of samples that chelate divalent cations include heparin and EDTA. $MgCl_2$ may be used to prepare samples for any endotoxin detection assay.
For your convenience, Certificates of Analysis are available online:
www.lonza.com/coa

www.lonza.com/accessories



Benefits
– Certified to contain less than 0.005 EU/mL endotoxin

2°C to 30°C (unopened)

Ordering Information – $MgCl_2$ 10 mM Solution

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
S50-641	S50-641	$MgCl_2$ 10 mM Solution	30 mL/vial	1 vial

Tris Buffer

Tris Buffer can be used in place of water as the sample diluent for highly acidic or basic samples (for endotoxin testing, samples and sample dilutions should be between pH 6–8 after lysate addition). Tris Buffer may be used to prepare samples for any of our endotoxin detection assays.

For your convenience, Certificates of Analysis are available online:
www.lonza.com/coa

www.lonza.com/accessories

Benefits
– Certified to contain less than 0.005 EU/mL endotoxin
– Certified pH range from 7.0 to 7.4 @ 25°C
– Certified to ensure good buffering performance

2°C to 8°C

Ordering Information – Tris Buffer 50 mM

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
S50-642	S50-642	Tris Buffer 50 mM	30 mL/vial	1 vial

Endotoxin and Endotoxin Challenge Vials™

Endotoxin (*E. coli*) Challenge Vials™ are for use in oven validation studies. Each vial contains >1,000 EU/vial. The vials may be tested using any of our endotoxin detection kits. Product 193783 contains high potency endotoxin and is intended for use in endotoxin removal system challenges, i.e. depyrogenation ovens, and other spiking studies. Each vial contains >1,250,000 EU/vial. E700 is the USP Reference Standard Endotoxin. Each vial contains 10,000 EU/vial.

For your convenience, Certificates of Analysis are available online:

www.lonza.com/coa

www.lonza.com/accessories

Benefits

– Products 192568 and 193783 are devoid of fillers

192568 and 193783 are stored at 2°C to 8°C

E700 storage conditions are -20°C

Ordering Information – Endotoxin and Endotoxin Challenge Vials™

Cat. No. NA	Cat. No. EU	Product Name	Product Description	Size
193783	193783	Endotoxin	> 1.25 million EU/vial	5 vials
192568	192568	Endotoxin Challenge Vials™	>1,000 EU/vial	25 vials
E700	E700	USP Reference Standard Endotoxin	10,000 EU/vial	1 vial

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10

Cell Services

Cell Services

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Introduction

Relevant Acceleration

We have a reputation for expertise in many areas of cell biology, from the earliest research through biopharmaceutical production. Our presence in many pharmaceutical product and service markets helps us to understand the limitations of existing technologies and guides our efforts to find and develop better solutions for our customers.

Lonza Research Solutions is the market leader in primary cell biology. Over the past decade, the use of primary cells has been expanding. As primary cells are more difficult to handle than cell lines, we deliver complete protocols to address this. The importance of consistent tools in drug discovery is indisputable, and we strive to standardize our highly relevant products.

Our Solutions

We focus on the following areas of product and service development.

- Primary cell supply, including diseased cells, stem cell-derived cells, iPSC generation and banking, immortalized cells, cells containing biosensors, transfection of difficult cells and cell expansion services
- Improved prediction from cell models, including contextual cell-based assays for toxicity and mechanism of action assessment
- Making it easier for you, including reagent production, Cells on Demand™ and Pluripotent Stem Cell Services are putting the assays you already use into biologically relevant primary cells

Our dedicated Drug Discovery Team gives you access to our expertise in many areas of cell biology. We trust that we have identified the products and services of value to drug discovery customers and we welcome the opportunity to discuss and develop them further with you.

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Cells on Demand™ Services

Cells on Demand™ was established to expand Lonza's current offering of quality primary cells. This service supports customers who require specialized cell products to meet their individual research needs and applications.

Cell Isolation Services

We offer modifications to existing Lonza primary cells and beyond. Let us utilize our vast tissue network to provide you with the specific cell type you need.

RAFT™ 3D Cell Culture Services

Lonza's patented RAFT™ 3D Cell Culture Systems is designed to closely mimic the *in vivo* environment of cells within 96- or 24-well plate formats. Utilizing Lonza's RAFT™ System, we can establish 3D Cell Culture Systems tailored to your needs.

Cell Characterization

Using Lonza-generated recombinant cell lines, or a specific primary cell type in 2D or 3D cell culture systems, cell-based assays can be developed for functional testing of recombinant proteins and small molecules.

Immortalization Services

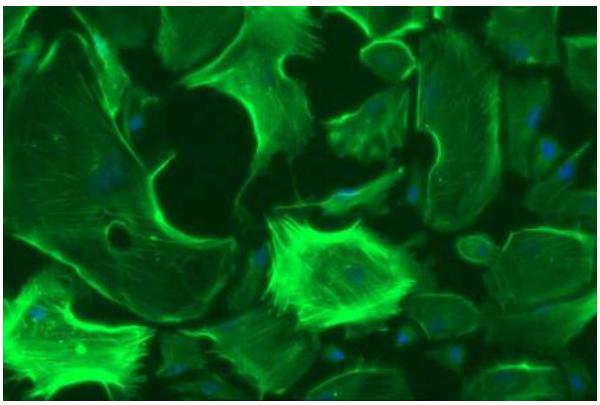
If your cell type of interest is not in our offering, the Cells on Demand™ team can produce an immortalized cell line to your specifications.

Generation of Recombinant Cells

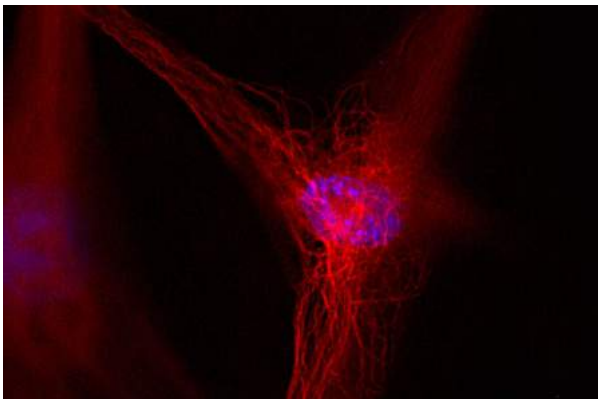
Utilize our extensive experience using difficult and hard to transfect cell lines to generate specific cell lines overexpressing your gene of interest.

Cell Line Expansion and Banking

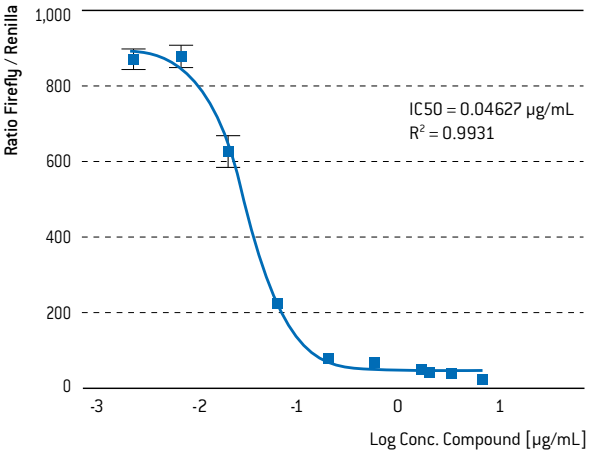
Cell line expansions are performed in accordance to your requirements and shipped to you in your preferred format, allowing you to focus on your assays and results.



Podocytes at P4, 10k a SMA overlay (20x)

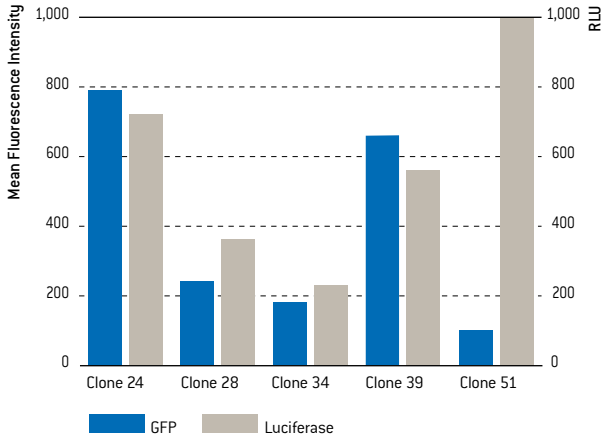


Dermal fibroblasts fixed and stained after 11 days in RAFT™ System.



Compound treament of sarcoma cells stably expressing an inducible luciferase reporter construct.

Double Stable Jurkat E6.1



GFP and luciferase expression level. Single-cell derived Jurkat E6.1 clones were generated by cotransfection of pmaxFP™-green N and a luciferase plasmid using Nucleofection and limited dilution.

www.lonza.com/cellsondemand

Clonetics™ Conditionally Immortalized Cell Lines

Cell lines are commonly used as screening models because production is easily scalable and these models minimize variability by providing uniform biological response over screening campaigns lasting many months. One disadvantage is that cell lines often lack the relevant phenotype of a cell *in vivo*. To address this shortcoming, we developed Clonetics™ Conditionally Immortalized Cells. These unique cells are generated by combining the telomere rejuvenating effects of telomerase with a specially modified temperature controlled T-antigen.

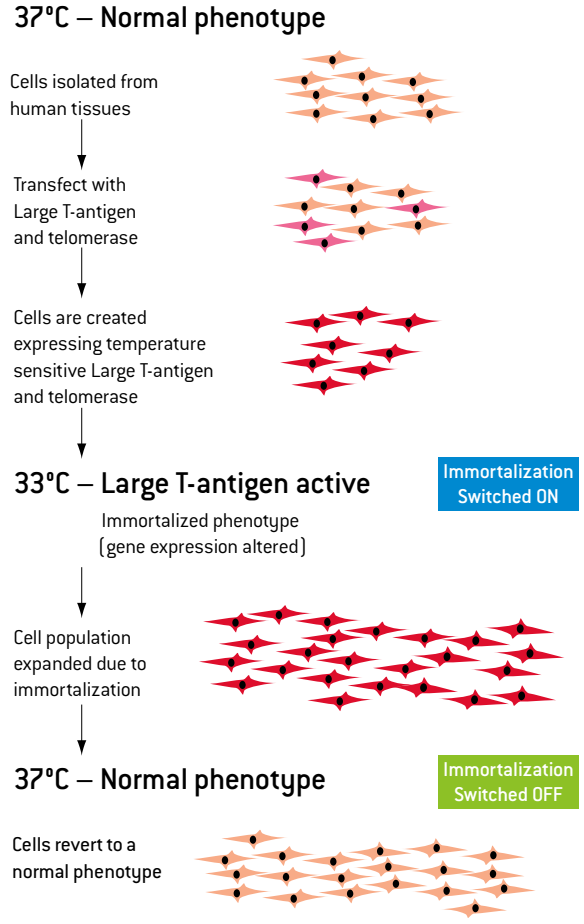
Benefits

- Biological relevance of primary cells
- Ease-of-use of a cell line
- Production is easily scalable
- Minimal lot-to-lot variation
- Unlimited supply of differentiated cells of same genotype

Applications

- High-throughput screening

Conditional Immortalization



Ordering Information – Cells

Cat. No.	Description	Proliferation Media	Media Cat. No.
XM13A1	Conditionally Immortalized Skeletal Muscle Myoblasts	SkGM™ 2	CC-3245
XM15B1	Conditionally Immortalized Skeletal Muscle Myoblasts	SkGM™ 2	CC-3245
XA15A1	Conditionally Immortalized Human Preadipocytes	SkGM™ 2*	CC-3245
XF05C1	Immortalized Human Dermal Fibroblasts	FGM™ 2	CC-3132
XS12C1	Immortalized Human Coronary Artery Smooth Muscle Cells	SmGM™ 2	CC-3182
XSEL6C1	Immortalized Human Lymphatic Microvascular Endothelial Cells	EGM™ 2MV	CC-3202
XSEB113C1	Immortalized Human Blood Microvascular Endothelial Cells	EGM™ 2MV	CC-3202
XSKA1B1	Conditionally Immortalized Human Adult Dermal Keratinocytes	KGM™ 2	CC-3107
00194607	Conditionally Immortalized Human Brain Microvascular Endothelial Cells	EGM™ 2MV	CC-3202

*Differentiation media PT-8002

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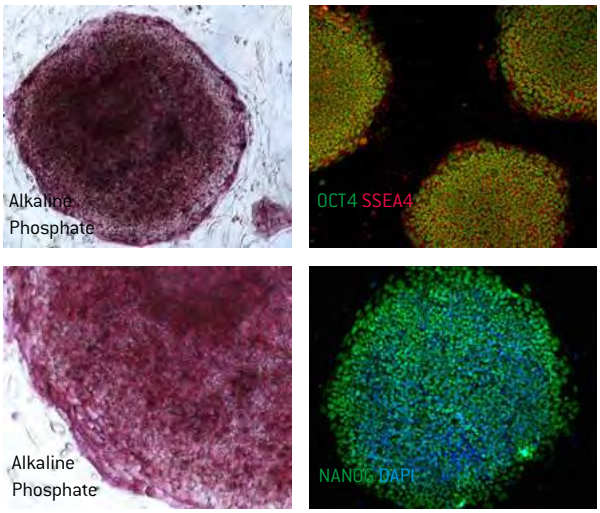
Pluripotent Stem Cell Services

Lonza established a new strategic vision to become the leading supplier to the regenerative medicine industry. To realize this vision, Lonza created the Pluripotent Stem Cell Innovation Center. Pluripotent stem cells (PSCs) have the ability to generate any of the 220+ cell types in the human body. And because of this unique attribute, these cells have great potential in basic research, drug discovery and cell replacement therapies.

PSC Services Lonza has built up expertise, capacity, and capabilities in pluripotent stem cell research and their application to cGMP manufacturing. Researchers can now access this expertise through our PSC service offering from iPSC generation to process development and differentiation.

Our services span the full value chain of pluripotent stem cells from tissue acquisition to differentiation:

- **Tissue Acquisition** – We have a dedicated team that procures both research and cGMP grade tissue according to the highest ethical standards and in compliance with government regulations
- **Reprogramming** – We offer cGMP and non-cGMP iPSC generation under feeder- and feeder-free conditions using a zero-footprint technology
- **Growth / Expansion / Banking** – We have established protocols using all of the common medium, matrix, and passaging methods. We also have the infrastructure and resources to support small- and large-scale culture and banking of PSCs
- **Characterization** – We offer all the standard methods of characterizing PSCs including thawing efficiency, mycoplasma and sterility testing, karyotype analysis, short tandem repeat genotyping, pluripotency marker expression (flow cytometry and immunofluorescence), and pluripotency assays (embryoid body and teratoma formation)
- **Differentiation** – We have established protocols for the production of PSC-derived motor neurons, dopaminergic neurons, and neural stem cells. We also have development programs underway to add to our differentiation portfolio of therapeutically relevant cell types
- **Process development** – Over the years we have built up expertise in the differentiation of high purity, functional cell types. Our team is well-versed in technology transfer and optimization of manufacturing protocols



Lonza iPSCs express hESC-associated markers POU5F1/OCT4 (green) and SSEA4 (red) counterstained with DAPI (blue).

Notes

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Testing Services

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QC Testing Solutions Services

Another means by which Lonza continues to strive to meet the needs of our QC microbiology customers is by providing complementary contract testing services for many of our QC microbiology related products.

QC Testing Solutions Services offered:

- Endotoxin Testing Services
- Recertification Services

QC Testing Solutions Services are presently operated out of two global Lonza facilities to provide our customers with worldwide access to our current service capabilities*:

- Lonza Walkersville, Maryland, USA
- Lonza Verviers, Belgium

*Not all testing services are offered in both locations.

Please contact your local sales representative or Customer Service for more information about where and how to submit samples to one of our service locations above.

Register for our QC Testing Solutions e-newsletter to stay up to date on the latest endotoxin detection products and services. Featured content includes technical tips, white papers and webinar events.

www.lonza.com/hsc

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Endotoxin Detection Testing Services

Lonza's Endotoxin Detection Testing Services offer you routine as well as customized endotoxin testing. Our facilities provide expertise in gel clot, kinetic chromogenic and kinetic turbidimetric LAL methods, as well as the rFC endpoint fluorescent assays.

USP, EP and FDA Compliant

All pharmaceutical grade endotoxin testing is performed in accordance with current regulatory documents such as the U.S. and European Pharmacopeia (USP and EP) Bacterial Endotoxins Test (BET), the 2012 U.S. Food and Drug Administration (USFDA) Guidance for Industry and the USP monograph for medical device testing. These documents originate from or are acceptable to the major Pharmacopeia and their regulatory authorities.

Whether you are from a university, research laboratory or a Quality Control laboratory in a major pharmaceutical company, Lonza has the capabilities you need for reliable, accurate and confidential results.

Benefits

- Market leader in endotoxin detection testing systems
- Technical expertise and full service reporting
- USP and EP compliant

STAT Testing Available*

For customers who may need faster turn-around times, Lonza offers an expedited testing service for preliminary screening, endotoxin determination, and the Endotoxin Challenge Vial™ test. Our STAT service delivers preliminary (unaudited) results in 4 days or less. STAT requests require prior approval from Lonza's Endotoxin Testing Services.

*STAT testing is only available in the US.

www.lonza.com/endotestserv

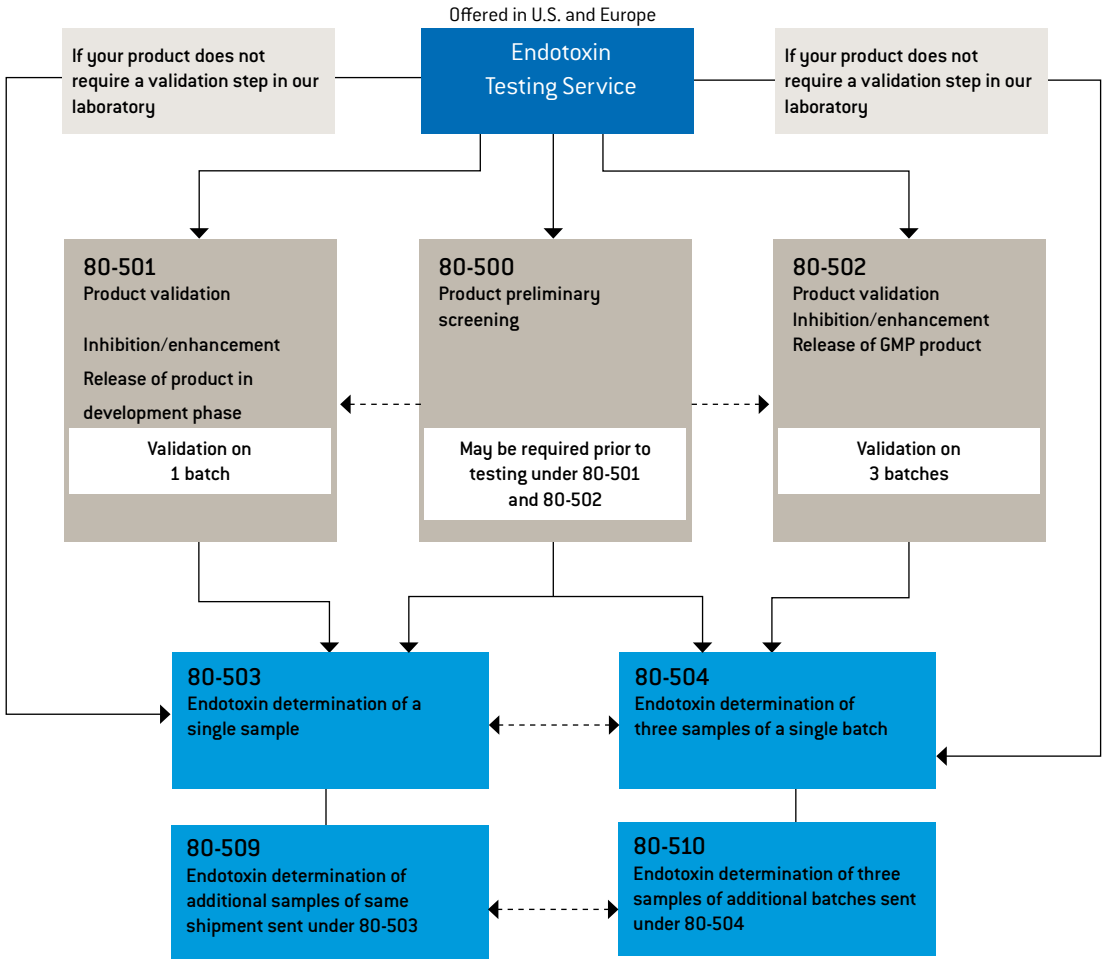

Figure 1. Endotoxin Testing Service.

Endotoxin Detection Testing Services

Continued

Pre-paid, Mail-in Anytime Testing Service

Available in Europe, the Endotoxin Testing Service offers a low cost mail-in anytime service for users that do not require testing according to pharmaceutical or medical device regulations.

This type of endotoxin testing is suitable for a wide range of non-standard tests such as:

- Samples from cell culture materials
- Samples of DNA plasmids
- Water samples from autoclaves or dialysis machines
- Dialysis water or fluids
- Research samples

Samples received using this service will be tested at the required dilutions indicated in the Pre-paid Endotoxin Testing Service diagram.

Benefits

- Low cost, convenient and quick endotoxin testing using Kinetic-QCL™ LAL Assay
- Quick, easy ordering process via web, phone, or fax
- Pre-addressed packaging for smooth delivery to Lonza's Endotoxin Testing Service

To utilize the Pre-paid Service, simply order one or more of the Rapid Endotest packs, place your sample in the sample containers provided, and mail the pre-addressed envelope back to Lonza.

When ordering the Pre-paid Service, the pre-paid endotest packs are for shipment of the samples to the Endotoxin Testing Services. When you have a requirement for an endotoxin test, simply mail in your samples. Samples will be tested and results sent to the customer within 5 days of receipt.

www.lonza.com/endotestserv

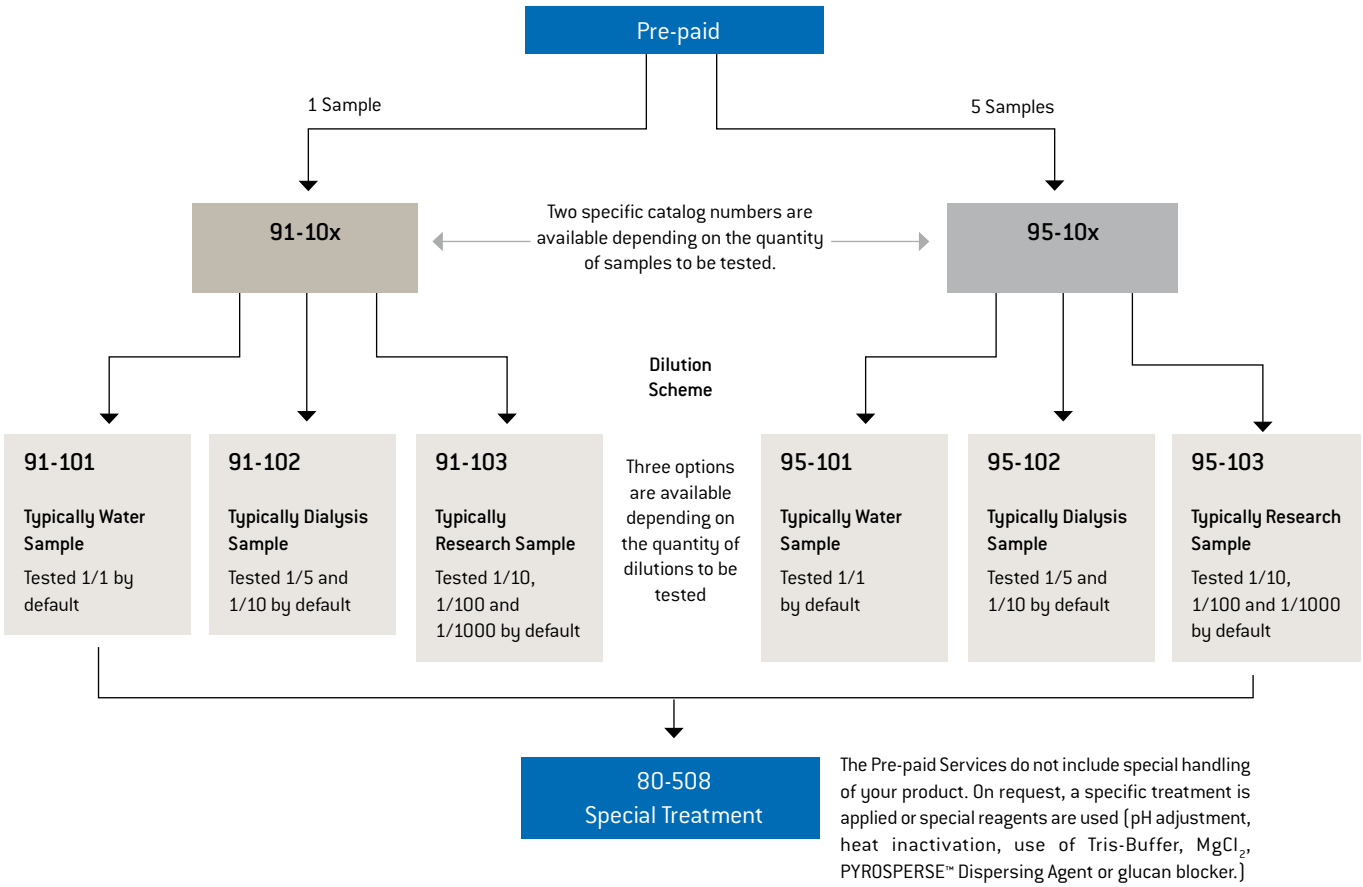

Figure 2. Pre-paid Testing Service, available in Europe.
This service is not avilable in the US.

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Endotoxin Detection Testing Services

Continued

Ordering Information – Endotoxin Detection Testing Services

Cat. No. NA	Cat. No. EU	Product Name	Product Description
80-500	BE80-500	Product Preliminary Screening	
80-501	BE80-501	Product Validation Inhibition/Enhancement	Release of product in development phase. Validation of one batch.
80-502	BE80-502	Product Validation Inhibition/Enhancement	Release of GMP product. Validation of three batches.
80-503	BE80-503	Endotoxin Determination of Single Sample	
80-509	BE80-509	Additional samples of same shipment sent under 80-503	
80-504	BE80-504	Endotoxin Determination of Three Samples of a Single Batch	
80-510	BE80-510	Endotoxin Determination of three samples of additional batches sent under 80-504	
80-508	BE80-508	Unusual sample treatment or handling charge per hour	
80-514	BE80-514	Exclusion of β-D-glucan activity testing	
80-535		Depyrogenation Endotoxin Challenge Vial™ Test	
80-544	BE80-544	Sample container screening	
80-500S		STAT: Product preliminary screening of one batch	
80-503S		STAT: Endotoxin determination of single sample	
80-509S		STAT: Additional samples of same shipment sent under 80-503	
80-504S		STAT: Endotoxin determination of three samples of a single batch	
80-510S		STAT: Endotoxin determination of three samples of additional batches sent under 80-504	
80-514S		STAT: Exclusion of β-D-glucan activity testing	
80-535S		STAT: Depyrogenation Endotoxin Challenge Vial™ Test	

STAT Testing is not available in Europe.

Ordering Information – Pre-paid Endotoxin Detection Testing Services

Cat. No. NA	Cat. No. EU	Product Name	Product Description
	BE91-101	Rapid-Endotest Single Water Sample	
	BE91-102	Rapid-Endotest Single Dialysis Fluid Sample	
	BE91-103	Rapid-Endotest Single Research Sample	
	BE95-101	Rapid-Endotest Five Water Samples	
	BE95-102	Rapid-Endotest Five Dialysis Fluid Samples	
	BE95-103	Rapid-Endotest Five Research Samples	
	80-515-X1	15 Endotoxin Challenge Vials™	Endotoxin testing of baked vials with positive product controls. Price includes ECVs.
	80-515-X2	20 Endotoxin Challenge Vials™	Endotoxin testing of baked vials with positive product controls. Price includes ECVs.
	80-515-X3	25 Endotoxin Challenge Vials™	Endotoxin testing of baked vials with positive product controls. Price includes ECVs.

Please contact your local Customer Service Team to obtain general information and the appropriate sample submission forms.

U.S. Endotoxin Testing Services
E-mail: scientific.support@lonza.com

European Endotoxin Testing Services
E-mail: scientific.support.eu@lonza.com

Recertification Services

Recertification Service for Stepped Neutral Density Test Plates

The Stepped Neutral Density (SND) and Absorbance (Universal) Test plates provide the ELx808™ Microplate Reader user with the ability to manage risk and help assure the reader is functioning properly between the recommended six-month preventive maintenance visits.
The ELx808™ Readers are robust instruments designed to provide years of reliable performance for endotoxin detection and other absorbance-based assays. In following current Good Manufacturing Practices (cGMP), the reader's performance should be verified and documented at regular intervals.

In order to properly assess the reader performance, the plates used in the testing must be shown to be acceptable. Plate manufacturers recommend the SND plate be recertified at least once per year.

Benefits

- Manages risk and helps ensure optimal performance of the ELx808™ Readers
- cGMP compliant processes, including testing of multiple parameters
- NIST-traceable testing conducted in an ISO 9001 certified facility

Parameters tested

- Absorbance accuracy (linearity and slope)
- Precision (reproducibility of results)
- Wavelength accuracy
- Optical alignment
- Detailed physical inspection

Reports include

- Physical inspection observations
- Coefficient of variation (%CV) for each plate standard filter
- Slope, R-value and Y-intercept linearity data
- Reader and plate uncertainty values
- Approval by a Quality Assurance representative
- Recertification due date (default is 12 months)

Please refer to the table below for additional details and ordering information. For testing parameters, reports and pricing, please contact your local sales representative.

www.lonza.com/recert

Ordering Information – Recertification Services

Cat. No. NA	Cat. No. EU	Product Name	Product Description
85-011-SND	85-011-SND	Stepped Neutral Density Plate Recertification Service	Two filter wavelengths 405 nm and 340 nm
85-012-SND	85-012-SND	Stepped Neutral Density Plate Recertification Service	Each additional filter wavelength
85-011BA	85-011BA	Stepped Neutral Density Plate Recertification Service	As Found and As Left testing at two filter wavelengths 405 nm and 340 nm
	85-013	Absorbance (Universal) Test Plate	Recertification Service*

SND plate recertification services offered for Lonza plate part number 25-342 only.
*Recertification services for BioTek Instruments, Inc., plate part number 7260522 (Absorbance Test Plate) are only available in Europe.

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Primary Cell Culture and Media

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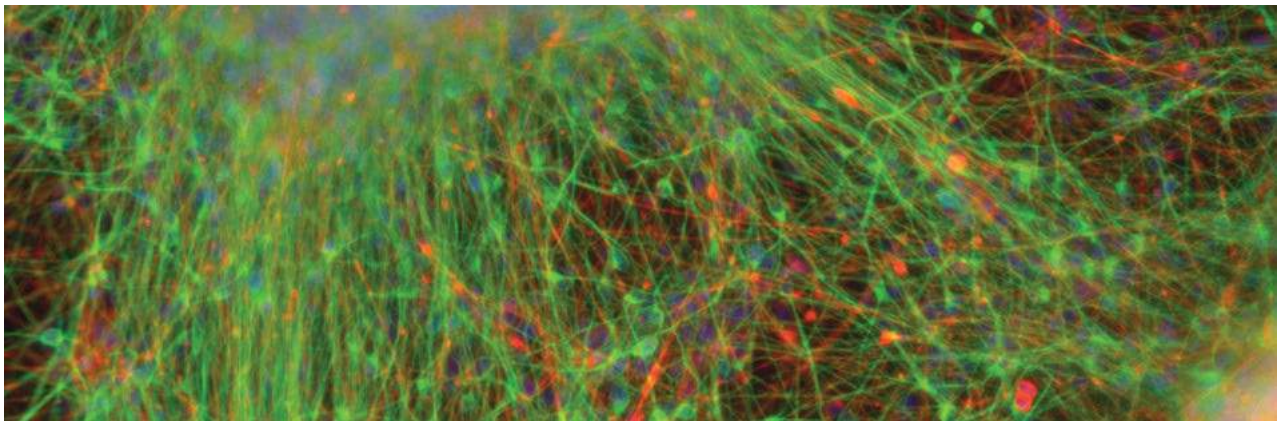

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Introduction

Lonza continues to set the standard for quality in the research industry with long-established and trusted brands for primary cells, media, transfection and separations. We also strive to lead the industry in Scientific Support for our customers. In addition to our global Scientific Support Team, we offer a broad selection of support tools for a variety of applications from basic discovery to applied research. The following chapter provides a selection of technical tips and guidelines to support your research and offers just a highlight of the large body of support materials available on our website.

Any technical advice or guidance furnished or recommendation by Lonza set forth herein is provided in good faith, but Lonza makes no warranty, either expressed or implied, as to its completeness or accuracy or the results to be obtained from use thereof. Any questions should be directed to Lonza at the contact information shown at the bottom of this catalog.

Primary Cells and Media

Whether you are using primary cells or cell lines in your research, we have product solutions and technical support materials to help your cell culture succeed. In addition to general cell culture workflow guidelines and media preparation instructions, we provide classical media formulations, thawing and set-up protocols, and instructions for serum-free media weaning.

Transfection

In this chapter we include the most critical guidelines for preparing cells for viable, high effeciency transfection. In addition to tips on substrate preparation and cell handling, we provide guidelines for successful siRNA experiments and generation of stable clones. For even further detail, our website offers a large collection of bench guides and white papers, created by our Scientific Support Team, discussing important considerations for successful transfection.

www.lonza.com/technical-library

Electrophoresis and Analysis

From detailed specifications on agarose and size markers, to specific instructions for DNA recovery and Western Blotting of proteins, we cover the basics needed to ensure successful separation, detection and analysis of nucleic acids and proteins in agarose and polyacrylamide gels. This section provides just a fraction of the comprehensive information available in our online Sourcebook for Electrophoresis.

www.lonza.com/sourcebook

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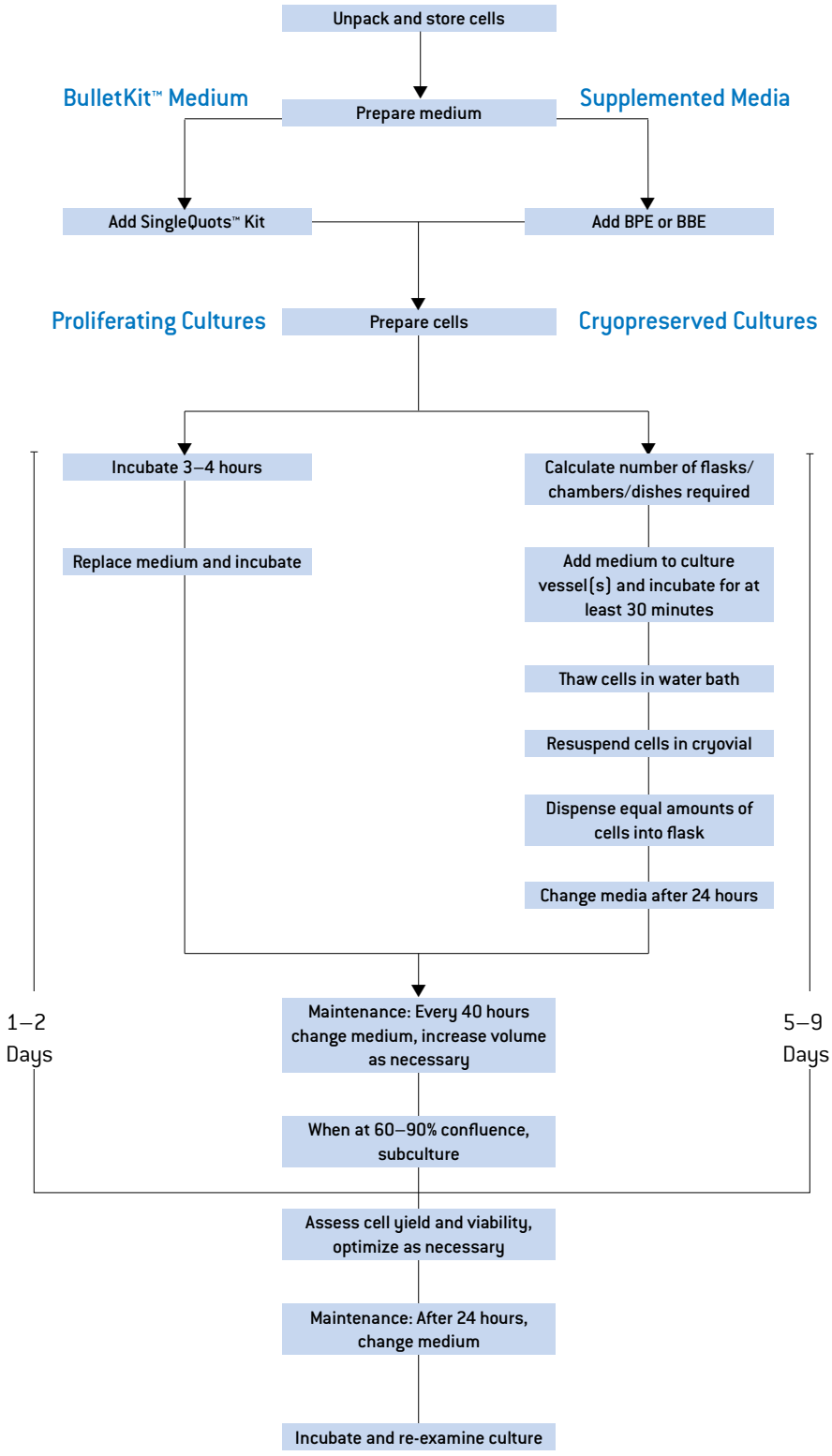
Overview of Cell Culture Process for Clonetics™ Human and Animal Cells*



Storage Requirements:

Cells

Upon arrival, immediately remove cryopreserved cells from dry ice and place immediately into liquid nitrogen. If no dry ice is left in the package, thaw cells, immediately place them into culture vessels and call your Scientific Support Specialist.

Medium

Store Clonetics™ Cell Culture Medium in a 4°C refrigerator. When using medium, under sterile conditions, take the amount you need and return bottle to the refrigerator. Always bring medium to room temperature before use.

Supplements and Reagents

If you to plan to subculture within three days, store all growth supplements, HEPES Buffered Saline Solution and Trypsin Neutralizing Solution at 4°C. Trypsin/EDTA Solution has a limited shelf life at 4°C. If, upon arrival, Trypsin/EDTA is thawed, immediately aliquot and refreeze at -20°C. If Trypsin/EDTA is frozen, store at -20°C. If you do not plan to set up the cell culture within 3 days, store all growth supplements and subculture reagents in a -20°C freezer.

*Exceptions: NHEM, rat and mouse neural cells, rat cardiac myocytes, h NHEPS™, pancreatic islets and InEpC Human Intestinal Epithelial Cells

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Safety Precautions with Clonetics™ Cells

- As a precaution against contamination, follow all procedures for handling products of human origin outlined in *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition (see link).
- Always wear gloves and safety glasses when working with all materials. Exercise caution when working with cryopreserved cells; rapid temperature changes may cause splattering of liquid nitrogen.
- Wash hands thoroughly after performing all procedures.
- Do not smoke, eat or drink in areas where reagents or cells are handled.
- Never pipette by mouth.
- Products of human and animal origin are potential biohazards. Although most provided human cells are tested and confirmed negative for HIV-1, Hepatitis B and C, proper precautions must be taken to avoid inadvertent exposure.

www.cdc.gov/biosafety/publications/bmbl5/

Caution: Clonetics™ and Poietics™ Products contain human sourced material. Treat as potentially infectious.

Media Preparation

Before You Begin

Perform the following steps before you begin media or cell preparation:

1. Prepare a sterile field.
   - A sterile field consists of a Class II biological safety cabinet with a front access opening and filtered laminar airflow, or an equivalent device
2. Determine the amount of medium required:
   - Review the Growth Area of Common Plasticware table (see page 399)to determine the amount of medium to be used
3. Sterile instruments and vessels required:
   - Sterile disposable serological pipettes
   - Micropipettes and sterile pipette tips
   - Adjustable multichannel pipette or repeating pipette
   - Sterile reservoirs for use with multichannel pipette
   - Sterile 15 mL centrifuge tubes
   - Cell culture flasks, or multiwell, flat-bottom tissue culture plates
   - Hemacytometer or cell counter
4. Other required supplies:
   - 70% alcohol (ethanol or isopropanol)
   - Growth medium (cell type specific)
   - Protective gloves and garments
   - Trypan Blue
5. Plan and prepare for initial set up:
   - Base the set-up on the number of cells indicated on the Certificate of Analysis accompanying the product
6. Check the calibration on the humidified incubator. Incubator should be humidified and set to 5% CO_2, 95% air, and 37°C.

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Media Preparation

Continued

Perform the Following Steps in a Sterile Field

For a bottle of fully supplemented medium, do the following:

1. Add Bovine Brain Extract (BBE) or Bovine Pituitary Extract (BPE), if required, to a 500 mL bottle of basal medium.
2. Detach the BBE or BPE supplement from the medium bottle.
3. Decontaminate the vial and medium bottle with ethanol or isopropanol.
4. Add the entire contents of the vial (approximately 2 mL) to the medium with a pipette; rinse the vial with medium and pipette the contents back into the 500 mL bottle.
5. Replace the cap and swirl the medium gently a few times to mix.
6. Record the date the BBE or BPE was added on the medium label. A fully reconstituted complete media should be used within 30 days; this supplemented medium will now be referred to as a growth medium.

For a BulletKit™ Medium, Perform the Following Steps

1. Decontaminate the external surfaces of the SingleQuots™ Cryovials and the basal media bottle with ethanol or isopropanol.
2. Aseptically open each cryovial and add the entire amount to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial; small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added; we recommend that you place the completed label over the basal medium label to avoid confusion or possible double supplementation.
5. Record the new expiration date on the label based on the shelf life. A fully reconstituted BulletKit™ Medium should be used within 30 days; this supplemented medium will now be referred to as a growth medium.

NOTE: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be re-filtered to assure sterility. If you re-filter, use a sterile 0.2-micron filter. Routine re-filtration is not recommended as some protein loss may occur with each filtration.

Growth Area of Common Plasticware

Flasks	Effective Growth Area	Cell Culture Medium Required	Initial Number of Cells to Seed at 2,500 cells/cm²	Initial Number of Cells to Seed at 3,500 cells/cm²	Initial Number of Cells to Seed at 5,000 cells/cm²
T-25	25 cm²	5 mL	62,500	87,500	125,000
T-75	75 cm²	15 mL	187,500	262,500	375,000
T-150	150 cm²	30 mL	375,000	525,000	750,000

Dishes	Effective Growth Area	Cell Culture Medium Required	Initial Number of Cells to Seed at 2,500 cells/cm²	Initial Number of Cells to Seed at 3,500 cells/cm²	Initial Number of Cells to Seed at 5,000 cells/cm²
35 mm	9.6 cm²	2 mL	20,000	28,000	40,000
60 mm	21 cm²	5 mL	52,500	73,500	105,000
100 mm	55 cm²	11 mL	137,500	192,500	275,000
150 mm	148 cm²	30 mL	370,000	518,000	740,000

Multiwell Plates	Effective Growth Area per well	Cell Culture Medium Required per well/total	Initial Number of Cells to Seed at 10,000 cells/cm²
6-well	9.60 cm²	2 mL / 12 mL	96,000
12-well	3.80 cm²	1 mL / 12 mL	38,000
24-well	2.00 cm²	0.5 mL / 12 mL	20,000
48-well	0.75 cm²	150 µL / 7 mL	7,500
96-well	0.32 cm²	100 µL / 10 mL	3,200

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Clonetics™ and Poietics™ Cell Culture Media

Medium Specifications

Medium is formulated for optimal growth of specific types of primary cells. It can be purchased as basal medium without supplements, as fully supplemented growth medium, or in a conveniently packaged BulletKit™ Medium that allows user control over supplement type and concentration. Each type of medium is tested for its ability to support growth or differentiation of the intended primary cell. Biochemical and sterility tests are also performed on every lot of medium. Certificates of Analysis are available for all medium products by visiting our website at:

www.lonza.com/coa

Basal Medium

Basal medium has been optimized for specific types of primary cells. Basal medium does not contain growth factors necessary for propagation of cells. Growth factors must be added to enhance plating efficiency and cellular proliferation. Optimized formulations make it possible to perform research on a wide variety of primary cell types.

Complete Medium

Complete medium is fully supplemented growth medium (BPE and BBE are packaged separately) and contains all of the growth factors and supplements necessary for the propagation of specific types of primary cells in culture. Undefined supplements are avoided when possible and used only at minimal levels when necessary. Standard formulations of all growth media include antimicrobials. Antimicrobial-free media are available as a custom order.

BulletKit™ Medium

A BulletKit™ Medium provides flexibility in final medium formulation and increased shelf life. Each BulletKit™ Medium contains basal medium and pre-measured, single-use aliquots (SingleQuots™ Kit) of growth factors and antimicrobial agents to formulate the fully supplemented growth medium of your choice.

Dental Pulp Stem Cell Media

Dental Pulp Stem Cell (DPSC) medium has been optimized for growth and proliferation of human dental pulp derived mesenchymal stem cells. When used together, Lonza's DPSC cells and media can quickly generate DPSC cultures for experimental studies, so you can spend time more time on gathering results.

DPSC BulletKit™ Medium

– Includes both the basal media and the necessary supplements for proliferation of human dental pulp derived mesenchymal stem cells.

DPSC Basal Medium

– Basal medium used to formulate the complete DPSC growth medium

DPSC SingleQuots™ Kits

– DPSC growth supplements used to formulate the complete DPSC growth medium

Product Information

Cat. No.	Product	Description
PT-3005	DPSC BulletKit™ Medium	500 mL DPSC Basal Medium plus PT-4105 SingleQuots™ Kit to formulate DPSC-GM (Growth Medium)
PT-3927	DPSC Basal Medium	Dental Pulp Stem Cell Basal Medium (500 mL)
PT-4516	DPSC SingleQuots™ Kit	Formulates 500 mL of DPSC Basal Medium to DPSC-GM Growth Medium; contains DPSC-GS (Growth Supplements), 50 mL; L-Glutamine, 10.0 mL; Ascorbic Acid, 5.0 mL; GA, 0.5 mL.

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Pulmonary Epithelial Cell Media

Pulmonary Epithelial cell media are serum-free media that have been optimized for the proliferation and differentiation of certain cells. Each component of the basal medium and each growth supplement are carefully titered for optimal growth by our R&D team. We offer many media choices for the growth of airway cells, allowing for desired performance and formulation flexibility. When selecting a medium to use, refer to specific media recommendations or call Scientific Support for assistance.

BEGM™ BulletKit™ Medium

– Best growth of NHBE and DHBE – Normal and Diseased Human Bronchial/Tracheal Epithelial Cells in medium containing antimicrobials

SAGM™ BulletKit™ Medium

– Superior growth for SAEC and D-SAEC – Normal and Diseased Human Small Airway Epithelial Cells

B-ALI™ BulletKit™ Medium

– Differentiation of Bronchial/Tracheal Epithelial Cells in an Air Liquid Interface culture

S-ALI™ BulletKit™ Medium

– Differentiation of Small Airway Epithelial Cells in an Air Liquid Interface culture

Product Information

Cat. No.	Product	Description
CC-3170	BEGM™ BulletKit™ Medium	Bronchial Epithelial Cell Growth Medium BulletKit™, Serum-free
CC-3171	BEBM™ Basal Medium	Bronchial Epithelial Cell Basal Medium, Serum-free
CC-4175	BEGM™ SingleQuots™ Kit	Formulates BEBM™ to BEGM™, BPE, 2 mL; Hydrocortisone, 0.5 mL; hEGF, 0.5 mL; Epinephrine, 0.5 mL; Transferrin, 0.5 mL; Insulin, 0.5 mL; Triiodothyronine, 0.5 mL; GA-1000, 0.5 mL; Retinoic Acid, 0.5 mL
CC-3118	SAGM™ BulletKit™ Medium	Small Airway Epithelial Cell Growth Medium BulletKit™, Serum-free
CC-3119	SABM™ Basal Medium	Small Airway Epithelial Cell Basal Medium, Serum-free
CC-4124	SAGM™ SingleQuots™ Kit	Formulates SABM™ to SAGM™, BPE, 2 mL; Hydrocortisone, 0.5 mL; hEGF, 0.5 mL; Epinephrine, 0.5 mL; Transferrin, 0.5 mL; Insulin, 0.5 mL; Triiodothyronine, 0.5 mL; GA-1000, 0.5 mL; Retinoic Acid, 0.5 mL; BSA-FAF, 5.0 mL
193514	B-ALI™ BulletKit™ Medium	Bronchial Air Liquid Interface BulletKit™, Serum-free The B-ALI™ BulletKit™ Medium includes a 250 mL bottle of B-ALI™ Growth Basal Medium, a 500 mL bottle of B-ALI™ Differentiation Basal Medium, and a B-ALI™ SingleQuots™ Kit
193515	B-ALI™ SingleQuots™ Kit	Formulates B-ALI™ Basal Media to growth and differentiation media, Transferrin, 0.9 mL; B-ALI™-Inducer, 1 mL; BPE, 3.3 mL; Epinephrine, 0.9 mL; GA-1000, 0.9 mL; hEGF, 0.9 mL; Hydrocortisone, 0.9 mL; Insulin, 0.9 mL; Retinoic Acid, 0.9 mL; Thiodothyronine, 0.9 mL
193516	B-ALI™ Growth Basal Medium	Bronchial Air Liquid Interface Basal Medium Growth, Serum-free
193517	B-ALI™ Differentiation Basal Medium	Bronchial Air Liquid Interface Basal Medium Differentiation, Serum-free
CC-4539	S-ALI™ BulletKit™ Medium	Small Airway Air Liquid Interface BulletKit™, Serum-free. The S-ALI™ BulletKit™ Medium includes a 250 mL bottle of S-ALI™ Growth Basal Medium, a 500 mL bottle of S-ALI™ Differentiation Basal Medium, and S-ALI™ SingleQuots™ Kit
CC-3281	S-ALI™ Growth Basal Medium	Small Airway Air Liquid Interface Basal Medium Growth, Serum-free
CC-3282	S-ALI™ Differentiation Basal Medium	Small Airway Air Liquid Interface Basal Medium Differentiation, Serum-free
CC-4538	S-ALI™ SingleQuots™ Kit	Formulates S-ALI™ Basal Media to growth and differentiation media, Transferrin, 0.9 mL; S-ALI™ Inducer, 1.0 mL; BPE, 3.2 mL; Epinephrine, 0.9 mL; GA-1000, 0.9 mL; hEGF, 0.9 mL; Hydrocortisone, 0.9 mL; Insulin, 0.9 mL; Retinoic Acid, 0.9 mL; Triiodothyronine, 0.9 mL; Bovine Serum Albumin-Fatty Acid Free, 8.0 mL

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Endothelial Cell Media

Endothelial cell media are low serum media optimized for the proliferation of endothelial cells. Each component of the basal medium and each growth supplement is carefully titered for optimal growth by our R&D team. We currently offer four Clonetics™ Media for the growth of endothelial cells allowing for desired performance and formulation flexibility. When selecting a medium to use, refer to specific medium recommendations or call Scientific Support for assistance.

EGM™ and EGM™ BulletKit™ Medium

- Basal medium developed for normal human endothelial cells in a low serum environment
- EGM™ Complete Medium is supplemented growth medium and includes an attached aliquot of Bovine Brain Extract (BBE)
- EGM™ BulletKit™ Medium includes basal medium with supplements and growth factors in separate, frozen aliquots
- Final serum concentration is 2%
- EGM™ Media can be used to grow all of Clonetics™ HUVECs isolated in EGM medium

EGM™ MV BulletKit™ Medium

- Developed for bovine microvascular endothelial cells
- Same basal medium as in EGM™ Media
- Final serum concentration is 5%

EGM™ 2 BulletKit™ Medium

- Refinements to basal medium and the growth factors
- Does not contain BBE
- Final serum concentration is 2%
- Improved cell proliferation over EGM™
- EGM™ 2 can be used to grow all of Clonetics™ Endothelial Cells except microvascular, coronary artery and iliac artery endothelial cells, and cells isolated in EGM™ Plus

EGM™ Plus BulletKit™ Medium

- Improved cell proliferation over EGM™
- No exogenous Vascular Endothelial Growth Factor (VEGF)
- Final serum concentration is 2%
- EGM™ Plus can be used to grow all of Clonetics™ HUVECs isolated in EGM™ Plus Medium

EGM™ 2MV BulletKit™ Medium

- Developed for the enhanced growth of microvascular, coronary artery and iliac artery endothelial cells
- Does not contain BBE
- Final serum concentration increased to 5%

Product Information

Cat. No.	Product	Description
CC-3125	EGM™ MV BulletKit™ Medium	Microvascular Endothelial Cell Growth Medium BulletKit™ Medium with 5% FBS
CC-4143	EGM™ MV SingleQuots™ Kit	Formulates EBM™ to EGM™ MV; BBE, 2 mL; hEGF, 0.5 mL; Hydrocortisone, 0.5 mL; FBS, 25 mL; GA-1000, 0.5 mL
CC-3202	EGM™ 2 MV BulletKit™ Medium	Microvascular Endothelial Cell Growth Medium-2 BulletKit™ Medium with 5% FBS
CC-4147	EGM™ 2 MV SingleQuots™ Kit	Formulates EBM™ 2 to EGM™ 2 MV; hEGF, 0.5 mL; Hydrocortisone, 0.2 mL; FBS, 25 mL; VEGF, 0.5 mL; hFGF-B, 2 mL; R3-IGF-1, 0.5 mL; Ascorbic Acid, 0.5 mL; GA-1000, 0.5 mL (No BBE)
CC-3162	EGM™ 2 BulletKit™ Medium	Endothelial Cell Growth Medium-2 BulletKit™ Medium with 2% FBS
CC-3156	EBM™ 2 Basal Medium-2	Endothelial Cell Basal Medium-2, Serum-free
190860	EBM™ 2 Basal Medium (1L)	Endothelial Cell Basal Medium-2 (1L)
CC-4176	EGM™ 2 SingleQuots™ Kit	Formulates EBM™ 2 to EGM™ 2; hEGF, 0.5 mL; Hydrocortisone, 0.2 mL; FBS, 10 mL; VEGF, 0.5 mL; hFGF-B, 2 mL; R3-IGF-1, 0.5 mL; Ascorbic Acid, 0.5 mL; GA-1000, 0.5 mL ; instead of , separating kit components; Heparin, 0.5 mL (No BBE)
CC-3024	EGM™ Complete Medium	Endothelial Cell Growth Medium with 2% FBS
CC-5035	EGM™-Plus BulletKit™ Medium	Endothelial Cell Growth Medium-Plus BulletKit™ Medium with 2% FBS
CC-5036	EBM™-Plus Basal Medium	Endothelial Cell Basal Medium-Plus, Serum-free
CC-4542	EGM™-Plus SingleQuots™ Kit	Formulates EBM™-Plus to EGM™-Plus; BBE, 1 mL; L-Glutamine, 25 mL; hEGF, 0.5 mL; Hydrocortisone, 0.5 mL; FBS, 10 mL; GA-1000, 0.5 mL ; Ascorbic Acid, 0.5 mL; Heparin, 0.5 mL
CC-3124	EGM™ BulletKit™ Medium	Endothelial Cell Growth Medium BulletKit™ Medium with 2% FBS
CC-3121	EBM™ Basal Medium	Endothelial Cell Basal Medium, Serum-free
CC-3129	EBM™-PRF	EBM™ – Phenol Red free
CC-4133	EGM™ SingleQuots™ Kit	Formulates EBM™ to EGM™; BBE, 2 mL; hEGF, 0.5 mL; Hydrocortisone, 0.5 mL; FBS, 10 mL; GA-1000, 0.5 mL ; Ascorbic Acid, 0.5 mL

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Fibroblast Cell Media

Fibroblast media has been optimized for the proliferation of fibroblasts. Each component of the basal medium and each growth supplement is carefully titered for optimal growth by our R&D team. We currently offer three media choices for the growth of fibroblasts, allowing for desired performance and formulation flexibility. When selecting a medium to use, refer to specific media recommendations or call Scientific Support for assistance.

FGM™ BulletKit™ Medium

– FGM™ is a defined medium system and does not contain serum

FGM™ 2 BulletKit™ Medium-2

– Contains a vial of FBS for a final serum concentration of 2%

FGM™ 3 BulletKit™ Medium-3

– Specially formualted for Cardiac Fibroblast growth. Contains a vial of FBS for a final serum concentration of 10%

FGM™ CD BulletKit™ Medium

– Supports isolation and proliferation of primary human dermal fibroblasts in culture
– Chemically defined – No serum and no animal or plant extracts
– Minimized variable experimental results due to unknown effects of animal or plant-derived components
– Obtain cleaner and more accurate results quickly

Product Information

Cat. No.	Product	Description
CC-3132	FGM™ 2 BulletKit™ Medium-2	Fibroblast Growth Medium BulletKit™ 2, with 2% FBS
CC-3131	FBM™ Basal Medium	Fibroblast Basal Medium
CC-4126	FGM™ 2 SingleQuots™ Kit	Formulates FBM™ to FGM™ 2; hFGF-B, 0.5 mL; Insulin, 0.5 mL FBS, 10 mL; GA-1000, 0.5 mL
CC-3130	FGM™ BulletKit™ Medium	Fibroblast Growth Medium BulletKit™
CC-3131	FBM™ Basal Medium	Fibroblast Basal Medium
CC-4134	FGM™ SingleQuots™ Kit	Formulates FBM™ to FGM™; hFGF-B, 0.5 mL; Insulin, 0.5 mL; GA-1000, 0.5 mL
CC-4526	FGM™ 3 BulletKit™ Medium-3	Fibroblast Growth Media BulletKit™ 3
CC-4525	FGM™ 3 SingleQuots™ Kit	Fibroblast Growth Media SingleQuots™ Supplements and Growth Factors for cardiac fibroblasts, Insulin, 0.5 mL; rhFGF-B, 0.5 mL; GA-1000, 0.5 mL; FBS, 50 mL
199041	FGM™ CD BulletKit™ Medium	Fibroblast Growth Medium BulletKit™ Medium – Chemically Defined
199019	FBM™ CD Basal Medium	Fibroblast Basal Medium – Chemically Defined
199020	FGM™ CD SingleQuots™ Kit	Formulates FBM™ CD to FGM™ CD; Growth Supplement, 5.0 mL

Hepatocyte Media

Product Information

Cat. No.	Product	Description
CC-3198	HCM™ BulletKit™ Medium	Hepatocyte Culture Medium, Phenol red-free
CC-3199	HBM™ Basal Medium	Hepatocyte Basal Medium, Phenol red-free, Serum-free
CC-4182	HCM™ SingleQuots™ Kit	Formulates HBM™ to HCM™; hEGF, 0.5 mL; Transferrin 0.5 mL; Hydrocortisone, 0.5 mL; BSA, 10.0 mL; Ascorbic Acid 0.5 mL; GA-1000, 0.5 mL; Insulin, 0.5 mL
CC-3197	HMM™ Medium	HMM™, Hepatocyte Maintenance Medium, Phenol red-free, Serum-free
CC-4192	HMM™ SingleQuots™ Kit	HMM™ SingleQuots™, required supplements for use with HMM™; to provide optimal maintenance of cells, Insulin, 0.5 mL; Dexamethasone, 0.5 mL; GA-1000, 0.5 mL

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International – Customer Service: +1 301 898 7025; Scientific Support: scientific.support@lonza.com

Keratinocyte Cell Media

Keratinocyte media has been optimized for the proliferation of keratinocytes. Each component of the basal medium and each growth supplement is carefully titered for optimal growth by our R&D team. We currently offer three media choices for the growth of keratinocytes, allowing for desired performance and formulation flexibility. When selecting a medium to use, refer to specific media recommendations in the cell systems sections or call Scientific Support for assistance.

KGM™ Gold BulletKit™ Medium

- Optimized for Clonetics™ NHEK – Normal Human Epidermal Keratinocytes in a serum-free environment
- KGM™ Gold BulletKit™ Medium includes basal medium with all supplements and growth factors in separate, frozen aliquots
- KGM™ Gold can be used to grow all Clonetics™ NHEK Normal Human Epidermal Keratinocytes and provides a nutrient rich medium that provides normal physiological growth characteristics and population doubling times

KGM™ 2 BulletKit™ Medium

- Supports proliferation of primary human keratinocytes in culture
- KGM™-2 BulletKit™ Medium includes the basal medium and supplements needed for growth

KGM™ CD BulletKit™ Medium

- Supports isolation and proliferation of primary human keratinocytes in culture
- Chemically defined – No serum and no animal or plant extracts
- Minimized variable experimental results due to unknown effects of animal or plant-derived components
- Obtain cleaner and more accurate results quickly

Product Information

Cat. No.	Product	Description
192060	KGM™ Gold BulletKit™ Medium	Keratinocyte Growth Media BulletKit™ Medium
192151	KBM™ Gold Basal Medium	Keratinocyte Basal Medium, 500mL
192152	KGM™ Gold SingleQuots™ Kit	Formulates KBM™ Gold to KGM™ Gold; Hydrocortisone; 0.5 mL; Transferrin; 0.5 mL, Epinephrine; 0.25 mL; GA-1000; 0.5 mL; BPE; 2.0 mL; hEGF; 0.5 mL; Insulin; 0.5 mL
195769	KGM™ Gold BulletKit™ Medium without Ca^{++}	Keratinocyte Growth Medium – Calcium-free and phenol red free BulletKit™
195130	KBM™ Gold Basal Medium without Ca^{++}	Keratinocyte Basal Medium – Calcium-free and phenol red free
CC-3107	KGM™ 2 BulletKit™ Medium-2	Keratinocyte Growth Medium-2 BulletKit™, Serum-free
CC-3103	KBM™ 2 Basal Medium-2	Keratinocyte Basal Medium-2, Serum-free
CC-3108	KGM™ 2 without Ca^{++} BulletKit™ Medium-2	Keratinocyte Growth Medium-2 BulletKit™, Calcium-free, Serum-free
CC-3158	KBM™ 2 without Ca^{++} Basal Medium	Keratinocyte Basal Medium-2, Calcium-free, Serum-free
CC-4152	KGM™ 2 SingleQuots™ Kit	Formulates KBM™ 2 to KGM™ 2; BPE; 2 mL; hEGF; 0.5 mL; Insulin; 0.5 mL; Hydrocortisone; 0.5 mL; Epinephrine; 0.5 mL; Transferrin; 0.5 mL; GA-1000; 0.5 mL
CC-4455	KGM™ CD BulletKit™ Medium	Keratinocyte Growth Media – Serum-free, Non-animal origin components
CC-3255	KBM™ CD Basal Medium	Keratinocyte Basal Media – Chemically defined
CC-4456	KGM™ CD SingleQuots™ Kit	Formulates KBM™ CD to KGM™ CD; Recombinant human insulin 1 mL; Growth supplement, 5 mL

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Mammary Epithelial Cell Media, Serum-free

Product Information

Cat. No.	Product	Description
CC-3051	MEGM™ Complete Medium	Mammary Epithelial Cell Growth Medium, Serum-free, Complete Medium
CC-3150	MEGM™ BulletKit™ Medium	Mammary Epithelial Cell Growth Medium BulletKit™, Serum-free
CC-3151	MEBM™ Basal Medium	Mammary Epithelial Cell Basal Medium, Serum-free
CC-3153	MEBM™ PRF	Mammary Epithelial Cell Basal Medium Phenol Red Free, Serum-free
CC-3152	MEBM™-Bicarb free	Mammary Epithelial Cell Basal Medium Sodium Bicarbonate Free, Serum-free
CC-4136	MEGM™ SingleQuots™ Kit	Formulates MEBM™ to MEGM™; BPE, 2 mL; Hydrocortisone, 0.5 mL; hEGF, 0.5 mL; Insulin, 0.5 mL; GA-1000, 0.5 mL

Melanocyte Cell Medium, Serum-free

Clonetics™ Melanocyte Cell Medium has been optimized for the growth and proliferation of normal human primary melanocytes in culture. This medium system has been shown to deliver superior results as compared to other existing commercial media systems. Melanocytes in culture maintain >90% functionality based on the conversion of L-dopa to dopa-melanin. The melanocyte media system also allows for normal morphology and proliferative capacity after recovery from cryopreservation and throughout serial passaging.

MGM™ 4 BulletKit™ Medium-4

- Melanocyte Growth Media has been qualified and tested together with NHEM to provide optimum performance
- The media system is offered as a BulletKit™ Medium (basal medium and separately packaged growth factors) to allow for flexibility with your research project
- Adult melanocytes also require the addition of Endothelin 3 (ET3) (CC-4510), sold separately.

Product Information

Cat. No.	Product	Description
CC-3249	MGM™ 4 BulletKit™ Medium	Melanocyte Cell Growth Medium BulletKit™ Serum-free
CC-3250	MBM™ 4 Basal Medium	Melanocyte Basal Medium, Serum-free
CC-4435	MGM™ 4 SingleQuots™ Kit	Formulates MBM™ 4 to MGM™ 4; $CaCl_2$, 1.0 mL; hFGF-B, 1.0 mL; PMA, 0.5 mL; rhInsulin, 1.0 mL; Hydrocortisone, 0.5 mL; BPE, 2.0 mL; FBS, 2.5 mL; Gentamicin/Amphotericin B, 0.5 mL

Neural Cell Medium, Low Serum

Product Information

Cat. No.	Product	Description
CC-3186	AGM™ BulletKit™ Medium	Astrocyte Growth Medium BulletKit™, with 3.0% FBS
CC-3187	ABM™ Basal Medium	Astrocyte Basal Medium, Serum-free, without L-Glutamine
CC-4123	AGM™ SingleQuots™ Kit	Formulates ABM™, to AGM™; Insulin, 1.25 mL; rhEGF, 0.5 mL; FBS, 15.0 mL; Ascorbic Acid, 0.5 mL; L-Glutamine, 5.0 mL; GA-1000, 0.5 mL

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Prostate Epithelial Cell Medium, Serum-free

Product Information

Cat. No.	Product	Description
CC-3166	PrEGM™ BulletKit™ Medium	Prostate Epithelial Cell Growth Medium BulletKit™, Serum-free
CC-3165	PrEBM™ Basal Medium	Prostate Epithelial Cell Basal Medium, Serum-free
CC-4177	PrEGM™ SingleQuots™ Kit	Formulates PrEBM™ to PrEGM™; BPE, 2.0 mL; Hydrocortisone, 0.5 mL; hEGF, 0.5 mL; Epinephrine, 0.5 mL; Transferrin, 0.5 mL; Insulin, 0.5 mL; Retinoic Acid, 0.5 mL; Triiodothyronine, 0.5 mL; GA-1000, 0.5 mL

Rat Cardiac Myocyte Medium

Product Information

Cat. No.	Product	Description
CC-4515	RCGM BulletKit™ Medium	Rat Cardiac Myocyte Growth Medium BulletKit™ Medium
CC-3275	RCBM Basal Medium	Rat Cardiac Myocyte Growth Basal Medium
CC-4516	RCGM SingleQuots™ Kit	Formulates RCBM to RCGM; Horse serum, 15 mL; FBS, 15 mL, GA-1000, 0.2 mL

Renal Cell Media, Low Serum

Product Information

Cat. No.	Product	Description
CC-3190	REGM™ BulletKit™ Medium	Renal Epithelial Cell Growth Medium BulletKit™, with 0.5% FBS
CC-3191	REBM™ Basal Medium	Renal Epithelial Cell Basal Medium, Serum-free
CC-4127	REGM™ SingleQuots™ Kit	Formulates REBM™ to REGM™; Hydrocortisone, 0.5 mL; hEGF, 0.5 mL; FBS, 2.5 mL; Epinephrine, 0.5 mL; Triiodothyronine, 0.5 mL; Transferrin, 0.5 mL; Insulin, 0.5 mL; GA-1000, 0.5 mL
CC-3146	MsGM™ BulletKit™ Medium	Mesangial Cell Growth Medium BulletKit™, with 5% FBS
CC-3147	MsBM™ Basal Medium	Mesangial Cell Basal Medium, Serum-free
CC-4146	MsGM™ SingleQuots™ Kit	Formulates MsBM™ to MsGM™; FBS, 25 mL; GA-1000, 0.5 mL

Retinal Pigment Epithelial Cell Medium

Product Information

Cat. No.	Product	Description
195409	RtEGM™ BulletKit™ Medium	Retinal Pigment Epithelial Cell Medium BulletKit™ Medium
195406	RtEBM™ Basal Medium	Retinal Pigment Epithelial Cell Basal Medium
195407	RtEGM™ SingleQuots™ Kit	Formulates RtEBM™ to RtEGM™; L-Glutamine, 4 mL; FBS, 4 mL; bFGF, 1 mL; GA-1000, 0.2 mL

Primary Neuron Growth Medium, Serum-free

Product Information

Cat. No.	Product	Description
CC-4461	PNGM™ BulletKit™ Medium	Primary Neuron Growth Medium BulletKit™ Medium
CC-3256	PNBM™ Basal Medium	Primary Neuron Basal Medium
CC-4462	PNGM™ SingleQuots™ Kit	Formulates PNBM to PNGM™; NSF-1, 4 mL; L-Glutamine, 2 mL; GA-1000, 0.2 mL
CC-4512	PNGM™-A BulletKit™ Medium	Primary Neuron Growth Media-Adult (contains CC-3256, CC-4462, and CC-4511)
CC-4511	PNGM™-A SingleQuots™ Kit	Formulates PNGM to PNGM™-Adult; OA, 0.5 mL; PA, 1.5 mL

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Human Mesenchymal Stem Cell Media

Product Information

Cat. No.	Product	Description
PT-3001	MSCGM™ BulletKit™ Medium	Mesenchymal Stem Cell Growth Medium BulletKit™ Medium
PT-3238	MSCBM™ Basal Medium	Mesenchymal Stem Cell Basal Medium
PT-4105	MSCGM™ SingleQuots™ Kit	Formulates MSCBM to MSCGM™ Growth Medium; MCGS, 50 mL; L-Glutamine, 10 mL; GA-1000, 0.5 mL
PT-3002	hMSC Differentiation Kit– Osteogenic	Mesenchymal Stem Cell Differentiation Kit – Osteogenic
PT-3924	hMSCBM – Osteogenic Basal Medium	Mesenchymal Stem Cell Basal Medium – Osteogenic
PT-4120	hMSC SingleQuots™ Kit – Osteogenic	Formulates Osteogenic Basal Medium to Osteogenic Differentiation Medium; Dexamethasone, 1 mL; β-Glycerophosphate, 2 mL; Ascorbate, 1 mL; Penicillin/Streptomycin, 2 mL; MCGS, 20 mL; L-Glutamine, 4 mL
PT-3003	hMSC Differentiation Kit – Chondrogenic	Mesenchymal Stem Cell Differentiation Kit – Chondrogenic
PT-3925	hMSCBM – Chondrogenic Basal Medium	Mesenchymal Stem Cell Basal Medium – Chondrogenic
PT-4121	hMSC SingleQuots™ Kit – Chondrogenic	Formulates Chondrogenic Basal Medium to Chondrogenic Differentiation Medium; ITS^{+}, 2 mL; Sodium Pyruvate, 2 mL; Proline, 2 mL; Dexamethasone, 1 mL; Ascorbate, 2 mL; GA-1000, 0.2 mL; L-Glutamine, 4 mL
PT-4124	TGF-β3	Required component sold separately
PT-3004	hMSC Differentiation Kit – Adipogenic	Mesenchymal Stem Cell Differentiation Kit – Adipogenic
PT-3102A	hMSC Adipogenic Maintenance Medium	Mesenchymal Stem Cell Maintenance Medium – Adipogenic
PT-3102B	hMSC Adipogenic Induction Medium	Mesenchymal Stem Cell Induction Medium – Adipogenic
PT-4122	hMSC Maintenance SingleQuots™ Kit – Adipogenic	Formulates Adipogenic MM to Adipogenic Differentiation Medium; rhInsulin, 2 mL; GA-1000, 0.2 mL; MCGS, 20 mL; L-Glutamine, 4 mL
PT-4135	hMSC Induction SingleQuots™ Kit – Adipogenic	Formulates Adipogenic M to Adipogenic Differentiation Medium; Indomethacin, 0.4 mL; IBMX, 0.2 mL; rhInsulin, 2 mL; Dexamethasone, 1 mL; GA-1000, 0.2 mL; MCGS, 20 mL; L-Glutamine, 4 mL
190632	MSCGM™ CD BulletKit™ Medium	Mesenchymal Stem Cell Growth Medium BulletKit™ Medium – Serum-free, Xeno-free
190620	MSCBM™ CD Basal Medium	Mesenchymal Stem Cell Basal Medium – Chemically Defined
192125	MSCGM™ CD SingleQuots™ Kit	Formulates MSCBM™ CD to MSCGM™ CD; Growth Supplement, 5.0 mL

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Human Adipose-Derived Stem Cell Medium

Product Information

Cat. No.	Product	Description
PT-4505	ADSC-GM BulletKit™ Medium	Adipose-Dervived Stem Cell Growth Medium BulletKit™ Medium
PT-3273	ADSC Basal Medium	Adipose-Derived Stem Cell Basal Medium
PT-4503	ADSC-GM SingleQuots™ Kit	Formulates ADSCBM to ADSC Growth Medium; FBS, 50 mL; L-Glutamine, 5 mL; GA-1000, 0.5 mL

Preadipocyte Growth Media

Product Information

Cat. No.	Product	Description
PT-8200	PGM™ Basal Medium	Preadipocyte Basal Medium
PT-8002	PGM™ 2 BulletKit™ Medium-2	Preadipocyte Growth Medium-2 BulletKit™ Medium
PT-8202	PBM™ 2 Basal Medium-2	Preadipocyte Basal Medium-2
PT-9502	PGM™ 2 SingleQuots™ Kit	Formulates PBM™ 2 to PGM™ 2; FBS (10%), 50 mL; L-Glutamine, 5 mL; GA-1000, 0.5 mL; rhInsulin, 2 mL; Dexamethasone, 0.2 mL; Indomethacin, 0.4 mL; IBMX, 0.2 mL

Osteoclast Growth Media

Product Information

Cat. No.	Product	Description
PT-8001	OCGM Growth Medium BulletKit™ Medium	Osteoclast Growth Medium BulletKit™ Medium
PT-8201	OCPBM Basal Medium	Osteoclast Basal Medium
PT-9501	OCGM Growth Medium SingleQuots™ Kit	Formulates OPBM to Osteoclast Growth Medium; FBS (10%), 10 mL; L-Glutamine, 1 mL; Penicillin/Streptomycin, 1 mL; M-CSF, 0.1 mL; Soluble RANK Ligand, 2 µg

Neural Progenitor Growth Media

Product Information

Cat. No.	Product	Description
CC-3209	NPMM™ BulletKit™ Medium	Neural Progenitor Maintenance Medium BulletKit™ Medium
CC-3210	NPBM™ Basal Medium	Neural Progenitor Basal Medium
CC-4241	NPMM™ SingleQuots™ Kit	Formulates NPBM™ to NPMM™ (for maintenance) rhEGF, 0.4 mL; rhFGF, 0.4 mL
CC-3229	NPDM™ BulletKit™ Medium	Neural Progenitor Differentiation Medium BulletKit™ Medium
CC-4242	NPDM™ SingleQuots™ Kit	Formulates NPBM™ to NPDM (for differentiation); NSF-1, 4 mL; GA-1000, 0.4 mL

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Skeletal Cell Media

Product Information

Cat. No.	Product	Description
CC-3207	OGM™ BulletKit™ Medium	Osteoblast Growth Medium BulletKit™, with 10% FBS
CC-3208	OBM™ Basal Medium	Osteoblast Cell Basal Medium, Serum-free
CC-4193	OGM™ SingleQuots™ Kit	Formulates OBM™ to OGM™; FBS, 50 mL; Ascorbi Acid, 0.5 mL; GA-1000, 0.5 mL
CC-4194	OGM™ Differentiation SingleQuots™ Kit	Induces osteoblast differentiation and bone mineralization; Hydrocortisone-21-Hemisuccinate, 0.5 mL; ß-Glycerophosphate, 5.0 mL
CC-3216	CGM™ BulletKit™ Medium	Chondrocyte Growth Medium BulletKit™ Medium
CC-3217	CBM™ Basal Medium	Chondrocyte Basal Medium
CC-4409	CGM™ SingleQuots™ Kit	Formulates CBM™ to CGM™; R3-IGF-1, 1.0 mL; bFGF, 2.5 mL; Insulin, 1.0 mL; Transferrin, 0.5 mL; FBS, 25 mL
CC-3225	CDM™ BulletKit™ Medium	Chondrocyte Differentiation Medium BulletKit™, Serum-free
CC-3226	CDM™ Basal Medium	Chondrocyte Differentiation Basal Medium
CC-4408	CDM™ SingleQuots™ Kit	Formulates CDM™ Basal Medium to CDM™ Differentiation Medium; TGF-β, 1.25 mL; R3-IGF-1, 0.5 mL; Insulin, 0.5 mL; Transferrin, 0.5 mL; FBS, 12.5 mL; GA-1000, 0.25 mL

Skeletal Muscle Cell Media

Product Information

Cat. No.	Product	Description
CC-3160	SkGM™ BulletKit™ Medium	Skeletal Muscle Growth Medium BulletKit™, Serum-free
CC-3161	SkBM™ Basal Medium	Skeletal Muscle Basal Medium, Serum-free
CC-4139	SkGM™ SingleQuots™ Kit	Formulates SKBM™ to SKGM™; hEGF, 0.5 mL; Insulin, 5 mL; BSA, 5 mL; Fetuin, 5 mL ; Dexamethasone, 0.5 mL; GA-1000, 0.5 mL
CC-3245	SkGM™ 2 BulletKit™ Medium-2	Skeletal Muscle Myoblast Growth Medium-2 BulletKit™, with 10% FBS
CC-3246	SkBM™ 2 Basal Medium-2	Skeletal Muscle Myoblast Basal Medium-2, Serum-free; no L-Glutamine
CC-3244	SkGM™ 2 SingleQuots™ Kit	Formulates SkBM™-2 to SkGM™ 2; FBS, 50.0 mL; GA-1000, 0.5 mL; rhEGF, 0.5 mL; Dexamethasone, 0.5 mL; L-Glutamine, 10.0 mL

Smooth Muscle Cell Medium

Product Information

Cat. No.	Product	Description
CC-3182	SmGM™ 2 BulletKit™ Medium-2	Smooth Muscle Growth Medium-2 BulletKit™ Medium with 5% FBS
CC-3181	SmBM™ Basal Medium	Smooth Muscle Basal Medium, Serum-free
CC-4149	SmGM™ 2 SingleQuots™ Kit	Formulates SmBM™ to SmGM™ 2; hEGF, 0.5 mL; Insulin, 0.5 mL; hFGF- B, 1 mL; FBS, 25 mL; GA-1000, 0.5 mL

Stromal Cell Medium, Low Serum

Product Information

Cat. No.	Product	Description
CC-3205	SCGM™ BulletKit™ Medium	Stromal Cell Growth Medium BulletKit™, with 5% FBS
CC-3204	SCBM™ Basal Medium	Stromal Basal Medium, phenol red-free, Serum-free
CC-4181	SCGM™ SingleQuots™ Kit	Formulates SCBM™ to SCGM™; hFGF-B, 0.5 mL; Insulin 0.5 mL; FBS, 25 mL; GA-1000, 0.5 mL

Procedure for Thawing Mononuclear Cells and Progenitor Cells

1. Warm medium containing 10% FBS or 1% BSA. For mononuclear cells and hematopoietic progenitors, DNase I (20 U/mL) should also be added.*
2. Quickly thaw the vial of frozen cells in a 37°C water bath. Wipe the outside of the vial with 70% ethanol.
3. Aseptically transfer a maximum of 2 mL of cell suspension to a 50 mL conical tube. For 1 million cells or less, use a 15 mL conical tube.
4. Rinse the vial with 1 mL of medium. Add the rinse dropwise to the cells while gently swirling the tube (≈1 minute).
5. Slowly add enough medium dropwise to the cells until the total volume is 5 mL, while gently swirling after each addition of several drops of medium (≈3 minutes).
6. Slowly bring the volume up to fill the tube by adding 1 mL to 2 mL volumes of medium dropwise, while gently swirling after each addition of medium (≈5–10 minutes).
7. Centrifuge the cell suspension at 200 × g at room temperature for 15 minutes.
8. Carefully remove most of the wash by pipette (and save in a second tube), leaving a few mLs behind so the cell pellet is not disturbed. Gently resuspend the cell pellet in the remaining medium. If you are using a 50 mL tube, transfer the cells to a 15 mL conical tube and rinse the 50 mL tube with 5 mL of medium. Slowly add the 5 mL wash medium to the cell suspension with gentle swirling.
9. Slowly bring the volume up to fill the tube by adding 1 mL to 2 mL volumes of medium while gently swirling after each addition of medium.
10. Centrifuge the cell suspension at 200 × g at room temperature for 15 minutes.
11. Carefully remove by pipette all but 2 mL of the wash. Gently resuspend the cell pellet in the remaining 2 mL of medium and count. If cell count is lower than expected, centrifuge the wash saved in step 8 at a higher speed, count and combine if necessary.
12. Rest the cells for 1 hour at 37°C and 5% CO_2. Count the cells a second time. The cells are ready to be put in toculture.

*For the addition of DNase, prepare 20 mL of medium containing 10% FBS and 20 U/mL of DNase I (Sigma D 4513). Proceed as above, using the DNase-containing medium to dilute the cells. Centrifuge the cells and continue with step 8.

Culture Set-up – Adherent Cell Types

These Instructions do not Apply to All Cell Types.

Please go to:
www.lonza.com/cell-protocols
for detailed, cell specific instructions.

1. Calculate the number of vessels to be set up. Refer to the Certificate of Analysis for the exact number of cells in the cryovial. Refer to table on page 399 "Growth Area of Common Plasticware", for help in adjusting this calculation.

   Use the following calculations to determine the number of vessels to be set-up for the recommended seeding densities of 2,500 cells/cm^2, 3,500 cells/cm^2, or 5,000 cells/cm^2

   $$\frac{\text{No. of cells available} \times \text{Percent viability}}{\text{Recommended seeding density}} = \text{Max. no. of } cm^2 \text{ that can be plated}$$

   $$\frac{\text{Max. no. of } cm^2 \text{ that can be plated}}{\text{Effective growth area of flask}} = \text{Max. no. of flasks that can be set up}$$

   Example: A cryovial of HMVEC-L with 520,000 cells and 80% viability

   $$\frac{520{,}000 \times 0.80}{5{,}000} = 83\ cm^2, \text{ to be set up}$$

   If you use a T-25 with an effective growth area of 25 cm^2

   $$\frac{83\ cm^2}{25\ cm^2} = \text{3 flasks (rounded down to the nearest whole number of flasks)}$$

   The advantage of setting up this number of T-25 flasks from the initial cryovial, as opposed to larger flasks, is that it reduces the risk of losing large numbers of cells. If you experience difficulty trypsinizing the first T-25 flask, there are other remaining T-25 flasks to use.
2. Label each flask with the passage number, cell type, lot number, and date.
3. In a sterile field, carefully open the supplemented bottle of growth medium and aseptically transfer the medium to new culture vessels by adding 1 mL growth medium for every 5 cm^2 surface area of the flask.
   Example: 5 mL growth medium for a 25 cm^2 flask.
4. Tighten vented caps on vessels. If vented caps are not being used, twist caps until tight, then loosen about 1/2 turn. Allow the culture vessels to warm and equilibrate in a 37°C, 5% CO_2, humidified incubator for at least 30 minutes.

Thawing Cells – Adherent Cell Types

Aseptically Add the Recommended Amount of Medium to the Flask and Equilibrate for 30 Minutes in a 5% CO_2, 37°C Incubator

1. Have a micropipette ready prior to thawing.
2. Remove the cryovial of cells from storage. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve the internal pressure, then retighten; do not open the cryovial completely.
3. Holding the cryovial, dip the bottom 3/4 of the cryovial in a 37°C water bath and swirl gently for 1–2 minutes until contents are thawed. Watch the cryovial closely; when the last sliver of ice melts remove it; DO NOT submerge it completely. Thawing the cells for longer than 2 minutes may result in less than optimal results.
4. Remove the cryovial immediately, wipe it dry, and transfer to a sterile field where the equilibrated flasks should be waiting, ready to seed. Rinse the cryovial with 70% alcohol, then wipe to remove excess.
5. Note the color of the thawed cryovial. Ideally, the color of the thawed cryovial should be pink. If the color is not pink, seed the cells, note the color and mention this fact to Scientific Support if seeding is not successful.

NOTES:

- If more than one cryovial is to be thawed, thaw one cryovial at a time and keep other cryovials in liquid nitrogen until ready for use
- Cryopreserved cells are very delicate; thaw and return them to culture as quickly as possible with minimal handling
- Wear eye protection when handling frozen cells; rapid temperature changes may cause splattering of liquid nitrogen
- Centrifugation should not be performed to remove cells from the cryoprotectant cocktail; this action is more damaging than the effects of DMSO residue in the culture
- It is not recommended to thaw frozen cells directly onto glass slides, chamber slides, gridded plates or multiwell plate configurations (6, 12, 24, 96…); optimal performance is achieved when initial seeding out of cryopreservation is performed into T-25 flasks; for further instructions follow the directions in the set-up section in the cell culture instructions provided or contact Scientific Support for cell-specific protocols

Seeding – Adherent Cell Types

Remove the Cap, Being Careful Not to Touch the Interior Threads with Your Fingers

1. Using a 1,000 µL micropipette set to 800 µL, put the tip into the cryovial and resuspend the cells with a gentle, slow and steady up and down pipetting motion no more than five times. DO NOT resuspend quickly and keep the tip near the bottom to avoid making bubbles.
2. Dispense an equal amount of cells into the flasks set up earlier (as determined by the recommended seeding density and number of cells/vial (see page 411)). If four T-25 flasks were prepared, set micropipette to 250 µL and dispense. If eight T-25 flasks were prepared, set micropipette to 125 µL and dispense.

   NOTE: Do not dispense the entire contents of the cryovial into one T-25 flask!

3. Replace the cap or cover and gently rock the vessels to evenly distribute the cells. Loosen caps if necessary to permit gas exchange.
4. Return the culture vessels to a 37°C incubator with 5% CO_2. Lay them flat on the shelf, providing the largest surface for cells to attach. The cells will anchor to the bottom surface of the flask.

After Seeding

Cells are not tolerant of rapid temperature fluctuations or nutrient-deficient medium. Feeding them with fresh growth medium that has been warmed will avert potential problems. (Remember to warm only the amount needed.) Check and feed the cells on the schedule below, even on weekends and holidays.

1. Change the growth medium the day after seeding (to remove residual DMSO and unattached cells), then every other day thereafter while examining them daily.

   NOTE: A change of medium requires removal of the medium by aspirating with a sterile pipette on the opposite side of the flask from where the cells are attached. Then warm, fresh medium is added down the same side.

2. Successfully recovered cultures will exhibit the following:
   2.1 Cells with clear non-granular cytoplasm.
   2.2 Numerous mitotic figures after day 2.
3. Feed the cells a larger volume of medium as they become more confluent. Use this table as a guideline:

If cells are:	Then feed them:
Under 25% confluent	1 mL per 5 cm^2
From 25–45% confluent	1.5 mL per 5 cm^2
Exceeding 45% confluence	2 mL per 5 cm^2

4. Continue feeding the cells until 60–90% confluence. If specific cell types are allowed to become over-confluent (i.e., epithelial cells) and stay at confluence for more than 2 days, they can suffer irreversible contact inhibition and may detach from the flask and/or be difficult to trypsinize.

Proliferating Cells – Adherent Cell Types

1. Examine the culture microscopically for any signs of distress during shipment (i.e., detachment, rounding-up, or atypical morphology). Check the relative cell density and estimate % confluency. The culture should be 30–100% confluent upon receipt. Some cellular detachment is normal. Please call Scientific Support immediately if cells look severely distressed.
2. Decontaminate the external surface of the cell culture flask or multiwell dish by wiping with 70% ethanol or isopropanol.
3. Incubate the sealed flask or multiwell dish at 37°C, 5% CO_2 for 3–4 hours to equilibrate temperature.
4. Warm an appropriate amount of growth medium to 37°C in a sterile container. Warming the entire bottle can shorten the shelf life of the medium. Never warm medium under hot running water or any other uncontrolled temperature source. **Never microwave.**
5. In a sterile field, carefully open the cell culture flask or multiwell dish, remove the medium and replace it with the warmed, fresh medium. Aseptically remove any medium inside the neck or cap area because it can facilitate microbial contamination.
6. If you are using a flask with a non-vented cap, loosen the cap and return the flask to the 37°C humidified incubator with 5% CO_2 for at least 24 hours.

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Subculturing – Adherent Cell Types

Storage Information for Subculture Reagents

1. Subculture reagents are sterile-filtered and then stored at -20°C until shipped from our Distribution Centers.
2. Subculture reagents may thaw during transport. They may be refrozen once.
3. Subculture reagents can be stored at -20°C until expiration date, after thawing once and refreezing.
4. To keep Trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and refreeze at -20°C. Trypsin/EDTA may be stored frozen until the expiration date.
5. We recommend that once the HEPES-BSS and the Trypsin Neutralization Solution are stored at 4°C they are used within one month.

Preparation

The following instructions are for a 25 cm^2 flask (T-25). Adjust all volumes accordingly for other size flasks.

Preparation for subculturing the first flask:

1. Subculture the cells when they are 60–90% confluent and contain many mitotic figures throughout the flask.
2. For each 25 cm^2 of cells to be subcultured:
   - 2.1 Thaw 2 mL of Trypsin/EDTA and allow to come to room temperature.
   - 2.2 Allow 7–10 mL of HEPES Buffered Saline Solution (HEPES-BSS) to come to room temperature.
   - 2.3 Allow 4 mL of Trypsin Neutralizing Solution (TNS) to come to room temperature.
3. Remove growth medium from 4°C storage and allow to start warming to room temperature.
4. Prepare new culture vessels and from step 4.3 on work in a sterile field:
   - 4.1 Prepare 1–3 T-75 flasks. The number of flasks needed depends upon confluence and total yield. Larger flasks may be used to save plasticware and time spent on subsequent subcultures. Smaller flasks reduce the risk of losing a substantial part of your culture.
   - 4.2 As before, label each flask with the passage number, lot number, cell type, and date.
   - 4.3 In a sterile field, carefully open the bottle and transfer growth medium to new culture vessels by adding 1 mL growth medium for every 5 cm^2 surface area of the flask.

   Example: 15 mL growth medium for a 75 cm^2 flask
   - 4.4 If not using vented caps, loosen caps of flasks. Place the new culture vessels into a 37°C humidified incubator with 5% CO_2 and equilibrate the flasks for at least 30 minutes. Subculture one flask at a time. All flasks following the first flask will be subcultured following an optimization of this protocol (explained later in this procedure), based on calculated cell count, cell viability, and seeding density.

NOTE: Use only Clonetics™ Trypsin/EDTA. The concentration of Trypsin/EDTA from other suppliers may be 10X Lonza's recommended concentration, which will detrimentally effect Clonetics™ Cells and will void Lonza's warranty.

In a Sterile Field

1. Aspirate the medium from one culture vessel.
2. Rinse the cells with 5 mL of room temperature HEPES Buffered Saline Solution (HEPES-BSS). DO NOT forget this step. The medium contains complex proteins that neutralize the trypsin.
3. Aspirate the HEPES-BSS from the flask.
4. Cover the cells with 2 mL of Trypsin/EDTA solution.
5. Tighten the cap and begin monitoring the flask under the microscope.
6. Continue to examine the cell layer microscopically.
   - 6.1 Allow the trypsinization to continue until approximately 90% of the cells are rounded up.

   NOTE: Rounded up cells are spherical, have smooth edges and are refractile or shiny. If the cells still have protruding nubs which are still attached to the flask, they need more time to trypsinize. This entire process takes about 2–6 minutes, depending on cell type.
   - 6.2 At this point, rap the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, you may not have let them trypsinize long enough. Wait 30 seconds and rap again. If cells still do not detach, wait and rap every 30 seconds thereafter.

   NOTE: Do not try to get all cells to detach by rapping them severely. This action may damage the cells.
7. After cells are released, neutralize the trypsin in the flask with 4 mL of room temperature Trypsin Neutralizing Solution. If the majority of cells do not detach within seven minutes, the trypsin is either not warm enough or not active enough to release the cells. Harvest the culture vessel as described above, and either re-trypsinize with fresh, warm Trypsin/EDTA Solution or rinse with Trypsin Neutralizing Solution and then add fresh, warm medium to the culture vessel and

Subculturing – Adherent Cell Types

Continued

return to an incubator until fresh trypsinization reagents are available.

8. Quickly transfer the detached cells to a sterile 15 mL centrifuge tube.
9. Rinse the flask with a final 2 mL of HEPES-BSS to collect residual cells, and add this rinse to the centrifuge tube.
10. Examine the harvested flask under the microscope to make sure the harvest was successful by looking at the number of cells left behind. This should be less than 5%.
11. Centrifuge the harvested cells at 220 × g for 5 minutes to pellet the cells.
    - 11.1 Aspirate most of the supernatant, except for 100 µL to 200 µL.
    - 11.2 Flick the centrifuge tube with your finger to loosen the pellet.
12. Dilute the cells in 4 mL to 5 mL of growth medium and note the total volume of the diluted cell suspension.

    NOTE: To obtain the best results from your cells, assess cell yield and viability with Trypan Blue.

13. Count the cells with a hemacytometer or cell counter and calculate the total number of cells. Make a note of your cell yield for later use.

    NOTE: The cell suspension should contain between 250,000 to 1,000,000 cell/mL for greatest accuracy.

14. If necessary, dilute the suspension with HEPES Buffered Saline Solution (HEPES-BSS) to achieve the desired "cells/mL" and re-count the cells.
15. Assess cell viability using Trypan Blue.
16. Use the following equation to determine the total number of viable cells.

    Total cell count × percent viability = Total # of viable cells
    Example: 1,000,000 cells × 60% = 600,000 viable cells

17. Determine the total number of flasks to inoculate by using the equation below. The number of flasks needed depends upon cell yield and seeding density. Larger flasks may be used to save plasticware and time spent on subsequent subcultures. Smaller flasks reduce the risk of losing a substantial part of your culture if contamination occurs.

The recommended seeding density could be 2,500 cells/cm^2, 3,500 cells/cm^2, or 5,000 cells/cm^2 for flasks and 10,000 cells/cm^2 for well plates

$$\frac{\text{Total \# of viable cells}}{\text{Recommended seeding density} \times \text{flask size}} = \text{Total \# of flasks to inoculate}$$

$$\frac{600{,}000 \text{ viable cells}}{75 \text{ cm}^2 \times 2{,}500 \text{ cells/cm}^2} = 3 \text{ T-75 flasks (rounded down to nearest whole number)}$$

18. Use the following equation to calculate the volume of cell suspension to seed into your flasks.

$$\frac{\text{Total volume of diluted cell suspension}}{\text{\# of flasks as determined in step 26}} = \text{Seeding volume}$$

$$\frac{4.3 \text{ mL of diluted cell suspension}}{3 \text{ T-75 flasks}} = 1.43 \text{ mL per T-75 flask}$$

19. Prepare flasks by labeling each flask with the passage number, lot number, cell type, and date.
20. Carefully open the medium bottle and transfer growth medium to new culture vessels by adding 1 mL growth medium for every 5 cm^2 surface area of the flask (1 mL/5 cm^2).

    Example: 15 mL growth medium for a 75 cm^2 flask.

21. After mixing the diluted cells with a 5 mL pipette to ensure a uniform suspension, dispense the volume of suspension calculated above into the prepared subculture flasks.
22. After dispensing the cells, gently rock flask to promote even distribution.
23. If not using vented caps, loosen caps of flasks. Place the new culture vessels into a 37°C humidified incubator with 5% CO_2.

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Subculturing into 96-well Plates

Overview

A culture flask of cells is harvested by trypsinization and subsequent trypsin inhibitor treatment. The cells are centrifuged, resuspended in growth medium and counted. The desired number of cells is then added to wells of sterile 96-well tissue culture plates. The plates are incubated in a 37°C, 5% CO_2 humidified incubator for 1–3 days to allow for cell adherence and growth. Seeding densities will vary somewhat with your experimental requirements. A density of 10,000 cells/cm^2 for multiwell plates is ideal.

Required Materials:

1. T-25 flask of proliferating cells between 60% and 90% confluence
2. 96-well flat bottom, tissue culture plates
3. 37°C humidified incubator with 5% CO_2 / 95% air
4. Laminar flow hood or other sterile environment
5. Adjustable multichannel pipette (8 or 12 channel) or repeating pipette
6. Sterile reservoir(s) for use with multichannel pipette

Procedure

1. Follow the steps for subculture preparation and subculturing. Then follow steps 8–10 below.
2. Since the cells/mL calculation computed is per mL, the cell concentration must be increased by 4 times before seeding 96-well plates (to accommodate the 1:4 dilution when adding 250 µL of suspended cells per well). When making the cell suspension, adjust the cell concentration with growth medium.
3. Transfer the diluted cell suspension to a sterile reservoir. Using a multichannel (8 or 12 channel) pipette equipped with sterile pipette tips, add 250 µL of the diluted cell suspension to each well of the labeled 96-well flat bottom, tissue culture plate(s).

   NOTE: Resuspend the cell suspension often during the seeding procedure to ensure a uniform number and distribution of cells into each well by pipetting up and down a few times between every other dispensing.

4. Cover and incubate the plates for 1–3 days at 37°C/5% CO_2. (Incubation periods exceeding 3 days are generally not recommended because of evaporation of medium from the edge wells of the plate).

   NOTE: Before using the 96-well plate culture in a bioassay, examine the cells microscopically for the presence of mitotic figures as a confirmation that the cells have resumed active growth.

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Instructions for Cryopreservation

Cryopreservation may compromise cell quality and performance. Performance of the cells cannot be guaranteed after cryopreservation.

Instructions

1. Sterile filter freezing medium using a cell culture rated 0.2-micron filter.
2. Harvest cells and spin them down.
3. Resuspend cells in the proper cold freezing solution (see below) at ≈500,000 to 2,000,000 cells/mL. Work quickly. Once exposed to DMSO, cells become very fragile.
4. Pipet aliquots (1 mL each) into freezing vials or ampoules and seal.
5. Insulate aliquots with a STYROFOAM® or propanol freezing canister.
6. Store cells at -70°C overnight.
7. Within 12–24 hours, place in LN2 (-196°C) for long-term storage. Cells will be compromised by prolonged storage at -70°C.

Clonetics™ Cell Cryopreservation Media Suggestions

Cell Type	Base Media	DMSO	FBS
General Clonetics™ Cell (see exceptions below)	80% Standard Growth Media	10% DMSO	10% FBS
Articular Chondrocytes (NHAC-kn)	80% CGM without FBS	10% DMSO	10% FBS
Melanocytes (NHEM)	60% MGM-4	10% DMSO	30% FBS
Osteoblasts (NHOst)	80% OGM without FBS	10% DMSO	10% FBS
Skeletal Muscle Cells (SkMC)	70% SkGM	10% DMSO	20% FBS
Skeletal Muscle Myoblasts (HSMM)	70% SkGM-2	10% DMSO	20% FBS

Poietics™ Cell Cryopreservation Media Suggestions

Cell Type	Base Media	DMSO	FBS/HSA	Hydroxyethyl Starch
General Poietics™ Cell (see exceptions below)	86.5% IMDM	7.5% DMSO	4% HSA (w/v)*	2% Hydroxyethyl starch (w/v)**
Adipose Derived Stem Cells (ADSC)	90% ADSC-GM	10% DMSO	No FBS/HSA	No Hydroxyethyl starch
Human Dental Pulp Stem Cells (DPSC)	92.5% DPSC-GM	7.5% DMSO	No FBS/HSA	No Hydroxyethyl starch
Human Mesenchymal Stem Cells (hMSC)	85% MSCBM	10% DMSO	5% HSA (w/v)*	No Hydroxyethyl starch
Preadipoytes (HPrAd)	80% EGM-2MV	10% DMSO	10% FBS	No Hydroxyethyl starch
Rat Mesenchymal Stem Cells (rMSC)	No Base Media	10% DMSO	90% FBS	No Hydroxyethyl starch

*If Human Serum Albumin (HSA) is not available, Bovine Serum Albumin (BSA) can be used at an equal w/v. If HSA and BSA are not available, Fetal Bovine Serum (FBS) may be used at 16% for General Poietics™ Cell or 20% for hMSC by reducing the amount of the base media appropriately.
**If Hydroxyethyl starch is not available, the component can be omitted by increasing the amount of IMDM to 88.5%

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Improving Cell Yield and Viability During Subculture

Several factors, or a combination of factors, can contribute to low cell count and low cell viability. If cell yield or viability is unsatisfactory, use the following information to increase the success rate of future cultures.

Improving Cell Yield

If your cell yield is low (less than 50%), determine the cause(s) and possible solution(s) using the table below. Then subculture one or more flasks applying the appropriate solution(s).

Low Yield (Cell Count)

Condition	Possible Causes	Solutions
Majority of cells did not detach	1. Inactive or cold Trypsin/EDTA	1. Use Trypsin/EDTA at room temperature
	2. Improper storage of Trypsin/EDTA	2. Store at -20°C until ready for use; thaw and allow it to come to room temperature briefly before subculturing
	3. Exposure time to Trypsin/EDTA was too short	3. Exposure time to Trypsin/EDTA is usually 5–6 minutes
	4. Trypsin/EDTA has been neutralized	4. Be sure to rinse the culture completely with HEPES-BSS before trypsinization
	5. Vessel was not rapped firmly	5. Use a moderate amount of force when rapping during trypsinization
Low yield, 95% of the cells detached but the yield was low	Culture was under confluent at trypsinization	Be sure to trypsinize at 60–90% confluence with numerous mitotic figures throughout the flask

Improving Cell Viability

If your cell viability is low (less than 50%), determine the possible cause(s) and solution(s) using the table below. Then subculture one more flask applying the appropriate solution(s).

Low Viability (<50% viable)

Condition	Possible Causes	Solutions
Trypsin/EDTA damaged the cells	1. Used another vendor's Trypsin/EDTA	1. Use only Clonetics™ Trypsin
	2. Exposure time of the cells to Trypsin/EDTA was too long	2. Do not trypsinize longer than 7 minutes
	3.Trypsin/EDTA was used above room temperature. Trypsin becomes more active at temperatures above room temperature	3. Do not use even mildly heated Trypsin/EDTA
	4. Failed to neutralize the trypsin. Prolonged exposure to trypsin will damage cells	4. Neutralize the Trypsin/EDTA with Trypsin Neutralizing Solution to eliminate cell damage due to trypsin
	5. Vessel was rapped too firmly during trypsinization. Rapping too hard to release cells causes cell membrane damage	5. Use moderate force when rapping flask to dislodge cells during trypsinization
Culture vessel was too confluent; was completely covered with cells	Culture was too confluent at trypsinization	Be sure to trypsinize at 60–90% confluence with about five mitotic figures per field of view
Cell growth slowed before 80% confluence and cells look dull and non-refractile	The most probable cause is failure to increase the volume of medium used as the cell confluency increased. The cells become mildly starved and are not able to recover after trypsinization	Change medium and increase volume as recommended. Please observe all guidelines

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Custom Cell Isolation Services

Consult the cell culture experts and harness the power of our extensive knowledge and experience delivering custom cell solutions. Choose from an endless variety of human and animal primary cell types which are cryopreserved, plated or in culture flasks, for your convenience. Save time and money by avoiding the aggravation of tissue acquisition, failed isolations and low yields.

Quality and Experience

As the cell culture experts, Lonza maintains an ISO 9001:2008 certified custom cell isolation laboratory staffed by some of the world's finest cell culture technicians, providing a variety of cell types for your individual research needs. Partner with us and benefit from over 60 years of combined cell culture isolation experience.

- **We offer:**
  - Expert custom cell isolation solutions made to your specifications
  - Extensive quality testing and cellular characterization available
  - Years of isolation experience with a wide variety of cell types
  - Custom formats: cryopreserved, plates or flasks

- **Delays in obtaining vital primary cell cultures can be very costly. Discover our superior custom cell isolation capabilities including:**
  - Primary cell isolations from human and animal tissues
  - Cell expansion and testing
  - Donor-matched cell sets
  - Cryopreserved or proliferating formats
  - Wide array of QC and cell characterization services, including PCR
  - Individual customer consultation to ensure correct order fulfillment

- **Custom primary cell isolations from human, rat, mouse, porcine, bovine, monkey and many other species. Examples of successful isolations include:**
  - Bovine adrenal gland capillary endothelial cells
  - Bovine embryonic kidney cells
  - Bovine and porcine preadipocytes
  - Canine umbilical vein endothelial cells
  - Cat iris smooth muscle cells
  - Guinea pig kidney cells
  - Human bladder epithelial cells
  - Human diabetic $CD34^+$ cells
  - Human microvascular retinal endothelial cells
  - Human small intestine disassociated cells
  - Murine dermal fibroblasts
  - Porcine mesangial cells

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Cell Culture Tips for Cell Lines and Primary Cells Prior to Transfection

Introduction

In order to help ensure that your cells are in the best possible condition before transfection, please take a moment to review the suggestions below. These are in no way intended to replace product protocols, but rather to give you some helpful hints to facilitate the success of your experiments.

Passage Number

Cells of a lower passage number typically respond better to transfection and will have higher transfection efficiencies and viabilities than those of higher passage numbers. For the most efficient gene transfer, we recommend using cells that are in logarithmic growth phase and at a passage number less than 10–15 (from the time of thaw). This is because some cell lines differentiate and change their features after many passages. If you are transfecting primary cells or cell lines that have been cryopreserved, we recommend that they be passaged at least two times to allow them to begin growing properly prior to transfection. Frozen primary blood cells should be incubated in growth medium for a minimum of 1–2 hours before transfection.

Growth Conditions

Adherent cells – For the transfection of adherent cells, the cells should be generally grown to a confluency of 70–85%. The confluency of the culture prior to transfection is important. If the cells are allowed to grow to a higher confluency than recommended, or up to 100% confluency, you may get transfection results other than what is stated in our protocols. We also suggest that cells be passaged 2–4 days prior to transfection, so that they achieve the required confluency for your experiments. For transfection of adherent cells by an electroporation-based method like Nucleofection (see page 168-241), cells had to be released from the culture vessel and transferred into specialized cuvettes. However with recent innovations of the Nucleofector™ Technology some cell types can now be transfected in adherence (see page 172 and 184).

An Important Note about Cancer Cells and Nucleofection
For the Nucleofection of cells from solid tumors, a primary cell kit can be used if the cells are less than passage 3. If the cells are passage 3 or higher, we recommend using a Cell Line Nucleofector™ Kit. For suspension cells, like leukemic cells, if the cells are less than passage 5, a Nucleofector™ Kit for primary cells can be used. If the cells are passage 5 or higher, we recommend using a Cell Line Nucleofector™ Kit.

Suspension cells – Suspension cells should be transfected when they are in the logarithmic growth phase. Generally, this corresponds to a density of 2–5 × 10^5 cells per mL. For Nucleofection of some cell types, a higher density is recommended. Please check the Optimized Protocol for the cell type you are using. The cells should be passaged 2–4 days prior to transfection so that they achieve the required density for your experiments.

For both adherent and suspension cells, it is important to make sure that the culture is growing properly and that the cells have the proper morphology. If they do not, this could indicate contamination with, for example, bacteria, fungi, or mycoplasma. Mycoplasma are common contaminants of cells grown in culture; studies indicate that between 5 and 35% of cultures are contaminated. Infections, which lead to many serious alterations in cell function and gene expression, are persistent and difficult to detect using conventional methods. We recommend our convenient 20-minute luminescent mycoplasma tests – MycoAlert™ or MycoAlert™ Plus Mycoplasma Detection Kit, (see page 161). If mycoplasma are detected, we strongly recommend discarding the cells. If the cells are truly irreplaceable, MycoZap™ Mycoplasma Elimination Reagent is a gentle, effective option (see page 163).

www.lonza.com/mycoplasma

Cell Harvesting

Proper cell handling during the harvesting process is crucial in order to maintain the health of the cell and helps ensure the success of your experiments. Before harvesting your cells, we recommend washing the monolayer to get rid of any residual growth medium, as well as calcium and magnesium ions. In most cases, PBS or HBSS without calcium or magnesium can be used. Other wash solutions can be used as well and will depend on the characteristics of the cell in use. For example, cultures that have multiple layers may detach easier if they are washed first with a 0.5 mM –1 mM EDTA solution or trypsin solution.

For Nucleofection in suspension, adherent cells will need to be detached from the culture vessel before they can be transfected. For cell lines, we recommend using trypsin at a concentration of 0.05% (0.5 mg/mL) and EDTA at 0.48 mM (0.2 mg/mL) in a balanced salt solution without calcium and magnesium.

Cell Culture Tips for Cell Lines and Primary Cells Prior to Transfection

Continued

Primary cells, like Clonetics™ Cells, should be treated more gently than cell lines. For example, we recommend using the Clonetics™ ReagentPack™ (CC-5034; see page 99), which contains a gentle trypsin solution, HEPES buffered saline, and trypsin neutralizing solution. Other dissociative enzymes can be used and again, this depends on the characteristics of the cell. For example, collagen rich cultures may require a collagenase dissociation. Please check with the cell supplier for specific recommendations.

Once the cells have detached from the growth vessel, inactivate the trypsin by adding either:

- Growth medium with serum
- Trypsin neutralizing solution from ReagentPack™ (CC-5034; see page 99)
- PBS/0.5% BSA

In all cases, it is important to monitor the cells during trypsin treatment because, if the trypsin is allowed to remain on the cells longer than necessary, it will damage the cell membranes resulting in high cell mortality.

Do not scrape the monolayer unless specifically recommended by the cell supplier. Scraping can cause mechanical damage to the cells and will not result in a single cell suspension.

When working with suspension cells, no detachment is necessary; simply spin down the required number of cells and remove as much residual growth medium as possible from the cell pellet. For Nucleofection, the cells will then be resuspended in Nucleofector™ Solution.

It is also important to avoid extra pipetting or unnecessary washing steps. Do not vortex your cells, as extra handling beyond what is recommended can potentially harm the cells and result in high cell mortality.

Helpful Hints for Adherent Cells

If you are working with a strongly adherent cell line, you can use a stronger trypsin solution. Solutions of 0.25% and 0.5% trypsin are routinely available from commercial suppliers. Alternatively, instead of washing the monolayer with PBS before the addition of trypsin, you can use a trypsin solution as the wash. Aspirate the trypsin, replace with fresh trypsin solution, and incubate until the cells detach.

If the cells are weakly adherent, you can wash with EDTA alone, which may be enough to detach the cells or you can try the following: wash cells with PBS, add trypsin and immediately aspirate off the trypsin solution. Incubate the cells with the residual solution until they detach. Alternatively, you can also wash the cells with ice cold PBS if the cells are particularly loose. In many cases, this alone may cause them to detach.

An Important Note About Lipids and Transfection

Most lipids cannot be used on suspension cells. It is important not to add antibiotics to the medium during transfection with lipids, as this will cause cell death. Antibiotics can be added to the growth medium after transfection. In some cases, it may be helpful to allow the cells to recover for 12–24 hours before adding any antibiotics.

Serum can be present in the growth medium after transfection and, in some cases, it can also be present in the transfection medium. Serum must not be present during complex formation because it inhibits the formation of the liposome complexes.

With lipids, it is also possible to scale up the transfection by varying the amounts of lipid, DNA, cells, and medium in proportion to the relative surface area of the culture vessel. Please check the protocol specific to your lipid for guidelines.

An Important Note About Centrifugation

For Nucleofection, it is important to follow the centrifugation guidelines as stated in the Optimized Protocols. Our standard for centrifugation is 90xg. We do not use RPM's because the speed you select in order to achieve 90xg will vary with the type of rotor in use in your lab. In order to determine the required speed to get 90xg, please consult the operation manual for your centrifuge or rotor.

If you do not have the manual, please visit the following link for a nomogram that you can use to convert g-forces to RPM:

http://www.geneinfinity.org/sp/sp_rotor.html

Alternatively, the correct rotor speed can be calculated by measuring the maximum radius of your rotor and entering the information into the table found on the Brinkmann website at:

www.sciencegateway.org/tools/rotor.htm

Centrifugation speeds and g-forces are not as critical with other transfection methods (i.e., standard electroporation, lipids) as they are with Nucleofection, which is why you will not see specific guidelines given in many protocols.

Cell Sources

For best transfection results, we recommend the use of cells with a known history, i.e., low passage number and free from contamination.

For primary cells, we recommend using Clonetics™ and Poietics™ Cells from Lonza, (see Chapter 1 and 2).

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Important Vector Factors for Gene Expression

Introduction

Our Scientific Support Team is commonly asked: "Why don't I get the same expression level of my gene if I use various vector backbones?" There are many components to a vector that can have an effect on the level of gene expression. Below you will find the 10 most important factors to consider when looking at vectors.

The selection of an appropriate expression vector is crucial for efficient gene expression. Just take a look at Figure 1. We tried 10 different vectors expressing the same luciferase gene in different backbones and expression cassettes and obtained highly variable expression levels.

Promoter Strength

Is your promoter appropriate for the cell type that you are working with? Table 1 describes the promoter strengths as a relative percent of the strength of the CMV promoter for various cells for which we have Optimized Protocols. The CMV promoter activity is set to 100% based on the CAT assay values from the referenced publications. Although CMV is a strong promoter in many mammalian cells, another promoter may give stronger expression in your cells (e.g., promoter SV40 in BHK-21 cells).

Introns

Many researchers consider constitutively spliced introns to be required for optimal gene expression; however, this point is not always agreed upon. The intron position and strength can affect transcription, mRNA export and polyadenylation. Thus, depending on its position, an intron can even lead to decreased gene expression.

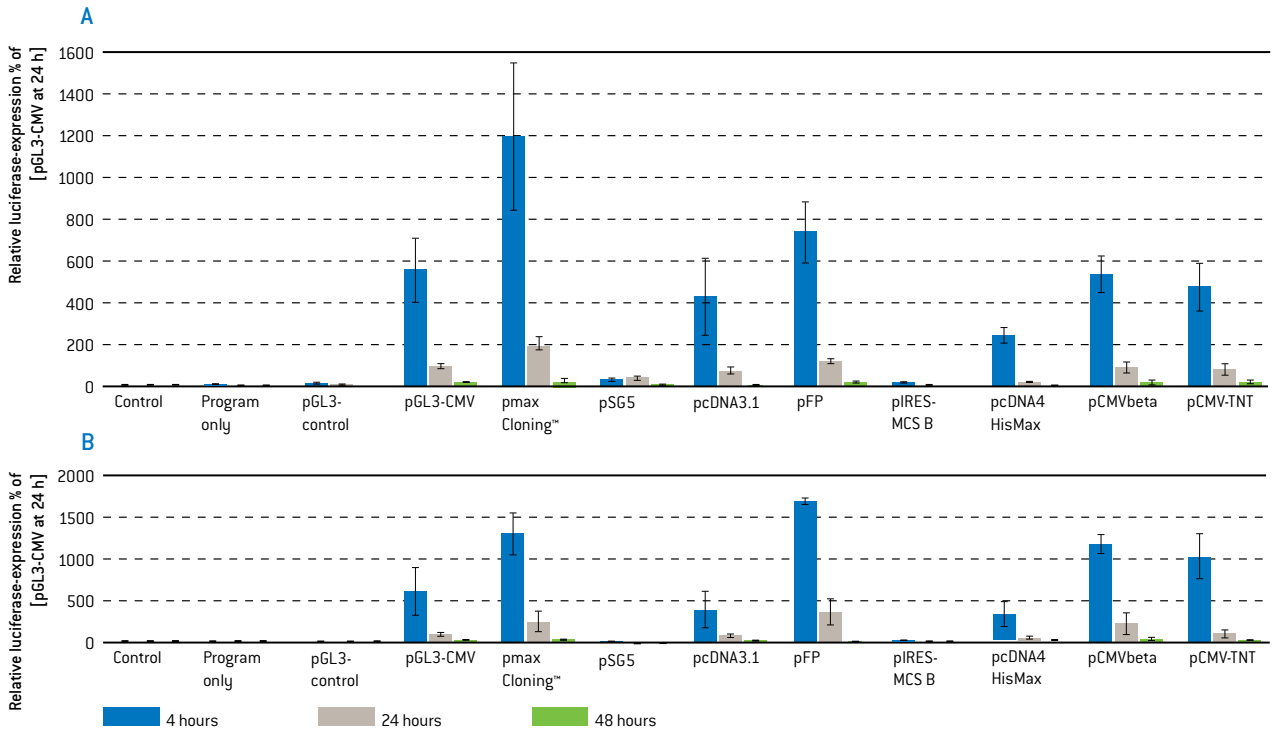


Figure 1. Luciferase expression levels depend on vector backbones. We looked at luciferase expression at 4, 24 and 48 hours in THP-1 and HUVEC cells. The amount of DNA was held at equimolar amounts based on plasmid size. For THP-1, the DNA amount ranged from 0.3–0.5 µg per reaction (A). For HUVECs, we used 2.5–4.4 µg of plasmid (B).

Important Vector Factors for Gene Expression

Continued

Table 1: Promoter Strengths in Different Cell Types

Cell Line	Source of Cells	SV40	EF1a	RSV	CMV	Reference
293	Human embryonic kidney	5%		74%	100%	4, 6
BHK-21	Hamster kidney	200%		200%	100%	4
C6	Rat glioma	44%			100%	5
CHO-K1	Chinese hamster ovary	16%		11%	100%	2, 4, 5
Cos-7	African green monkey kidney	7%	15%	7%	100%	1, 2, 4, 5, 8
HeLa	Human cervical carcinoma	43%	73%	29%	100%	2, 3, 4, 5, 6, 8
N2A (Neuro-2A)	Murine neuroblastoma	50%			100%	5
NIH-3T3	Murine fibroblast	67%	143%	107%	100%	2, 3, 7, 8

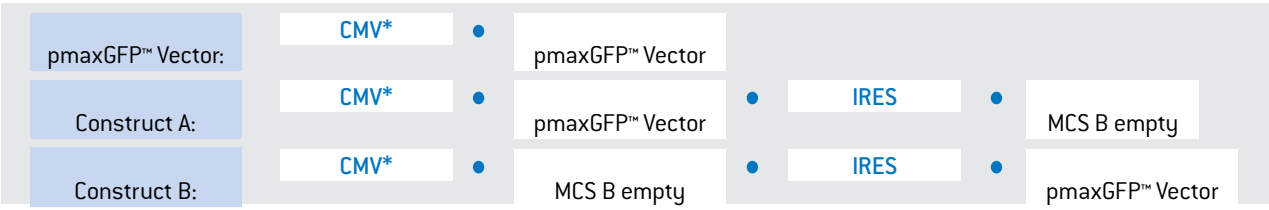
IRES Plasmids

With IRES plasmids, the promoter drives the expression of two genes, the cloned gene of interest (usually the upstream gene), and a reporter gene, often encoding a GFP protein (the downstream gene). The mRNA expressed from an IRES plasmid is a bicistronic message, meaning that both genes are present on the same mRNA molecule. Equal amounts of the messages which encode each gene are present in the mRNA population. However, the translation initiation efficiency of the two genes differs significantly. Ribosome binding to the initiation region of the upstream gene is very efficient, while the IRES allows ribosome binding and translation initiation for the downstream gene often at a significantly lower level. Since the downstream gene is usually GFP, the expression level of GFP will be lower than it would be normally seen when compared to plasmids without an IRES-sequence. Equal amounts of the protein of interest and GFP can be obtained using a fusion of both proteins, constructs containing two expression cassettes, or co-transfection.

We looked at pIRES expression vectors (Clontech) with a reporter (GFP) cloned in either the Multiple Cloning Site (MCS) upstream of the IRES (construct A) or downstream of the IRES (construct B; Figure 2).

Figure (2B) demonstrates that GFP expression is

A



B

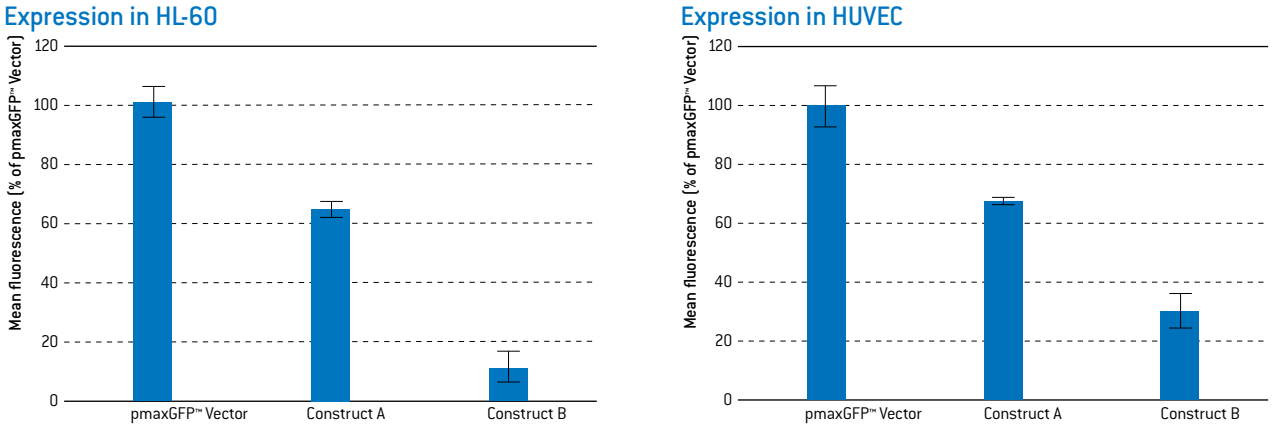


Figure 2. Reporter gene expression is dependent on the position in an IRES expression vector. HL-60 and HUVEC cells were transfected by Nucleofection with either pmaxGFP™ Vector or pIRES variants containing maxGFP™ Reporter Protein cloned in either the Multiple Cloning Site (MCS) upstream (construct A) or downstream of the IRES sequence (construct B; 2A). Figure (2B) shows reduced GFP expression using IRES plasmids especially if GFP is located downstream of the IRES sequence.

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Important Vector Factors for Gene Expression

Continued

drastically reduced if GFP is located downstream of the IRES. This study is shown for the GFP reporter gene, but was also done using luciferase with similar results.
Does your plasmid contain an IRES sequence? If so, where is it located? Keep in mind that the stability of the bicistronic mRNA can be influenced by either of the inserted genes. The levels of expressed protein for the first and second genes will not be identical, and this can create problems with analysis and interpretation. As a result, the true efficiency of the plasmid can be underestimated due to the lower expression level of your reporter. Be sure to use a very sensitive detection method for the reporter gene down stream of the IRES.

LTR (Viral Long Terminal Repeats)

Some expression plasmids utilize promoters and enhancers obtained from the Long Terminal Repeats (LTRs) of retroviruses, and when these expression plasmids are transfected into certain cells, the expression of the cloned genes might be suppressed by the cell. Although the mechanism of suppression is not completely understood, it is likely that plasmids containing promoters or enhancers derived from retroviral LTRs will not function well in primary cells and some cell lines. As Nucleofection is often the only effective method for primary cells, such suppressive effects might be observed more frequently with Nucleofection. The only effective alternative can be to reclone the gene of interest into a different expression plasmid that uses conventional promoters such as CMV (e.g., pmaxCloning™ Vector), EF1a or SV-40.

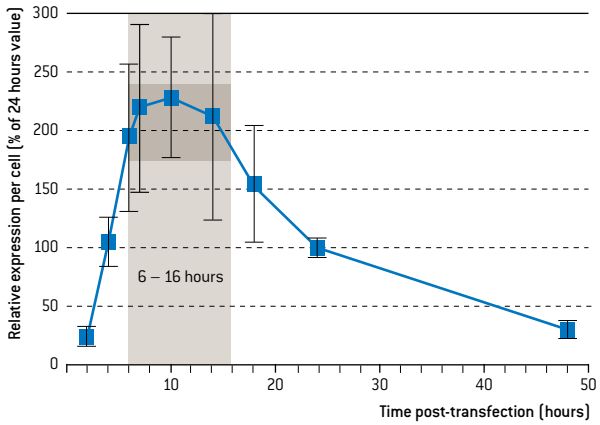
Size of Vector

We routinely use plasmids of 4–7 kb in our laboratories and Nucleofection of plasmids up to approximately 20 kb can be achieved. Using plasmids larger than this will most likely result in lower transfection efficiency. The general rule is that the larger a plasmid, the more difficult it becomes to get it inside the cell. This is true for electroporation or lipid-mediated transfections Nevertheless, some preliminary results using Nucleofection indicate that BAC's can be transfected as well but also with low transfection efficiency.[9, 10]

Reporter

What kind of reporter are you using? It needs to be safe, reproducible, quantitative, and sensitive. Your reporter should not be expressed by the cell endogenously at high levels and should function well with your downstream assays. When you change reporters, or if you change transfection methods, the kinetics of that reporter's expression using the current transfection method need to be evaluated to make sure that you are analyzing at the optimal time point. Luciferase, for example, has very different expression kinetics, depending on whether the transfections are being done by Nucleofection (maximum expression at 6–16 hours post-transfection) or lipids (maximum expression at 24 hours post-transfection). We found that luciferase kinetics are related to the transfection method and not to the vector backbone or cell type tested. Since kinetics of reporter expression also depend on mRNA and protein stability, we then compared the kinetics of luciferase expression to that of β–gal expression

Kinetics of Luciferase Expression in HUVEC



Kinetics of ß-gal Expression in HUVEC

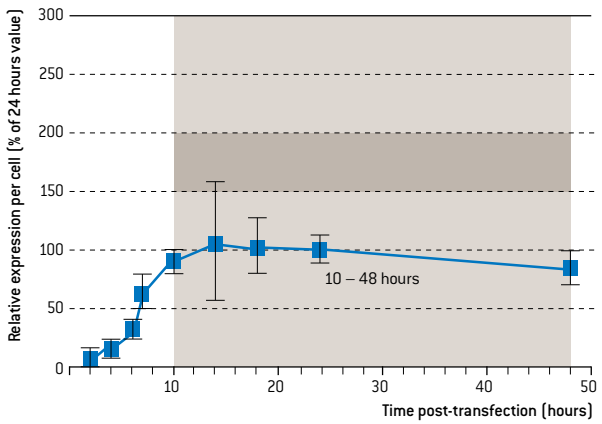


Figure 3. Expression kinetics are reporter gene dependent. HUVEC cells were transfected by Nucleofection with either a luciferase or a ß-gal expression vector. While luciferase expression shows a maximum 6–16 hours post-transfection, ß-gal expression is sustained for several days after transfection. Values represent relative protein amounts per well, normalized to 24 h value set to 100%.

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Important Vector Factors for Gene Expression

Continued

following Nucleofection (Figure 3). These data are from a co-transfection of HUVEC cells with a luciferase vector and a ß–gal vector. Both reporters can be under-represented at very high levels. However, the kinetics of expression for each reporter are very different. Luciferase has a very pronounced drop-off of expression after 16 hours. However, the ß–gal expression reaches a maximum at 10 hours post-transfection and levels off. If a single time point for analysis is chosen, such as 24 hours, the maximum expression of luciferase will be missed and again can under-represent the efficiency of that vector. As a consequence, we recommend performing luciferase analysis 6–16 hours post Nucleofection, whereas the optimal analysis time point for ß–gal or GFP expression is after 10–48 hours post Nucleofection.

Detection Methods

The detection method is predetermined by the reporter. Some reporters can be measured in multiple ways. GFP, for example, can be read by a fluorescent microscope, flow cytometer or a fluorescent plate reader. If only a qualitative picture is needed, the fluorescent microscope can provide a cost effective option. When looking for quantitative data, a flow cytometer or fluorescent plate reader should give more accurate data.

Fusion Vectors vs. Co-Transfections

Expression of a fusion protein depends on the localization of the protein, transcription and translation, as well as the folding and stability of the fusion protein. To improve expression, it may also be advisable to change the terminus to which the protein is fused. Co-transfections can be used instead of a fusion vector. One plasmid would contain the reporter gene (i.e., GFP) and the second plasmid would contain the gene of interest to be expressed. Depending on differences in promoter strength and vector size, the ratio of the two vectors needs to be optimized.

Hairpin Structures in Gene Product

A hairpin structure that forms in the RNA can affect translation of the gene. This should be considered, for example, when introducing mutations into the gene to be expressed.

Kozak Sequence

The Kozak sequence can slow down the rate of scanning by the ribosome and improve the chance of it recognizing the start of translation at the ATG start codon. If the Kozak sequence is contiguous with the ATG start codon, it can greatly increase the efficiency of translation and the overall expression of the gene of interest.

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Preparation and Quality

The quality of DNA used for transfection plays a central role for the success of the experiment.

We strongly recommend the use of high quality products for plasmid purification, e.g., Qiagen® EndoFree® Plasmid Kits. The purified DNA should be resuspended in sterile deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) before use. It has been demonstrated that DNA which was not purified via an endotoxin-free method can result in poor cell viability for some cell types. The same effect can be observed when endotoxin is added to clean DNA preparations. We do not recommend using phenol:chloroform or other organics in the preparation of the DNA as these are toxic to living cells and very difficult to remove completely.

For cells which are sensitive to activation by lipopolysaccharides, such as monocytes, macrophages and dendritic cells, an additional purification step via PEG precipitation is helpful. DNA should be dissolved in water. To 100 µL DNA in water, add 750 µL 5.0 M NaCl and 750 µL 40% w/v PEG 8000. Mix the contents of the tube by inverting several times and incubate on ice for one hour. Spin at top speed in a microcentrifuge for 15 minutes at 4°C. Remove supernatant, dissolve pellet in 100 µL water and repeat PEG precipitation. Carefully remove supernatant. Rinse the pellet with 500 µL ice cold 70% ethanol. Spin 3 minutes. Remove supernatant. Air dry the pellet and resuspend in 20 µL sterile water or TE (adapted from Molecular *Cloning: A Laboratory Manual (Third Edition)* by Joseph Sambrook, Peter MacCallum Cancer Institute, Melbourne, Australia; David Russell, University of Texas Southwestern Medical Center, Dallas).

Measuring Quality and Concentration of DNA

DNA purity should be measured by the ratio of absorbance (A) at 260 and 280 nm. The A260/A280 ratio should be at or above 1.6 for transfection use. Additionally, the plasmid should be run on an agarose gel to check for any nicked DNA or degradation. At least 90% of the DNA should be in the supercoiled conformation and no degradation products should be visible. To determine concentration, measure the absorbance at a wavelength of 260 then calculate as follows:

A260 × 50 µg/mL × dilution factor = DNA concentration.

Be sure that the dilution used is in the linear range of the spectrophotometer, usually an OD of 0.1–1.0. If using a microcuvette with a path length of less than 1 cm, it will be necessary to multiply by the factor to convert to the OD of a 1 cm path. For example, the path length of the 5 µL cuvette is only 0.5 mm or 1/20 cm, so it would require multiplying the above formula by 20 to get the concentration.

Optimal DNA Amounts for Nucleofection

Gene transfer efficiency can also be affected by the amounts of DNA. For Nucleofection of most cell types, we start with 1–2 µg DNA per 100 µL reaction with our pmaxGFP™ Vector which is ~4 kb in size. For larger constructs, it may be necessary to add higher amounts of DNA, so we recommend titrating the DNA to see if increasing amounts are helpful. The plasmid amounts can be increased up to 10 µg per sample or more in some cases. However, certain cells are sensitive to DNA and, in those cases, more DNA will result in increased mortality of the cells. If the Optimized Protocol for a cell type recommends using less than 2 µg of pmaxGFP™ Vector, those cells are likely DNA sensitive.

NOTE: The DNA concentration should be such that no more than 10 µL of substrate per 100 µL reaction is added in order to not dilute the Nucleofector™ Solution too far or exceed the tolerance of the cuvette, which could result in an error on the device.

Working with Highly Diluted DNA

In order to keep the total DNA volume to add to a Nucleofection reaction in the appropriate range, it may be necessary to ethanol precipitate your DNA if it is too diluted. An ammonium acetate-based ethanol precipitation followed by two 70% ethanol washes should ensure that there is minimal salt carry over. The procedure is to add 0.5 volumes of 7.5 M ammonium acetate and 2 volumes ethanol to the DNA in solution and mix well. Spin at full speed in a microcentrifuge for 15 minutes. Carefully remove supernatant. Rinse the pellet with a volume equal to the precipitation of ice cold 70% ethanol. Spin for 5 minutes. Remove supernatant. Repeat. Air dry the pellet and resuspend in sterile water or TE. Generally an assumption of about 70% recovery is good for determining the volume to resuspend. Then read A260 to confirm.

Background

Stable, long-term expression of a gene of interest can be either achieved by eukaryotic vectors that harbor elements for episomal maintenance in the nucleus of a transfected cell or via direct integration of the transfected plasmid into the target cells genome. Episomal stability is often limited, resulting in gradual loss of transfected vectors that can only be prevented by sustained antibiotic selection eliminating cells that lost the plasmid. Furthermore, the functionality of episomal plasmid elements is often restricted to certain species. Although integration into the host cell chromosome is a rare event and, for most purposes, clonal events have to be isolated, stability of the intended genetic modification usually is much higher.

Initially, the gene of interest has to be introduced into the cell (A), subsequently into the nucleus (B), and finally, it has to be integrated into chromosomal DNA (C) (Figure 1).

Since chromosomal integration into host chromosomes is a rare event, stably-transfected cells usually have to be selected and cultured in various ways. For the selection of stably-transfected cells, a selection marker is co-expressed on either the same construct or on a second, co-transfected vector. A variety of systems for selecting transfected cells exists, including resistance to antibiotics, such as neomycin phosphotransferase, conferring resistance to G418, dihydrofolate reductase (DHFR), or glutamine synthetase (Southern and Berg, 1982). After gene transfer, cells are cultivated in medium containing the selective agent. Only those cells which have integrated the plasmid containing the drug resistant gene survive.

Several options are used for the generation of a stable cell line, depending on the scope of the experiment (see Table 1). A mixed population of drug resistant cells can be used directly for experimental analysis (batch culture) with the advantage of generating fast results, but also the disadvantage of dealing with an undefined and genetically mixed cell population. To generate clonal cells, it is necessary to dilute the resistant cells in such a way that culture as single, isolated cells is achieved e.g., by plating in 96-well plates or other methods. Subsequently, the selection process is applied to the single cell cultures. The procedure of single cell cloning may be repeated several times to obtain 100% clonal purity. This culture method allows for conduction of the study or the screening using a defined and homogenous cell system. So far, generation of stable cell lines has been a major challenge for many cell types (e.g., Jurkat, MCF7 or U937) since overall transfection efficiencies and/or integration frequencies have been low. While common transfection methods, such as lipofection, can be used for the stable expression in easy-to-transfect cell lines (e.g., HeLa, COS-7 or CHO), Nucleofection is the method of choice for stable expression in difficult-to-transfect cell types.

The generation of stably-transfected cell lines is essential for a wide range of applications, such as gene function studies (Grimm, 2004), drug discovery assays or the production of recombinant proteins (Wurm, 2004). In contrast to transient expression, stable expression allows long term, as well as defined and reproducible, expression of the gene of interest.

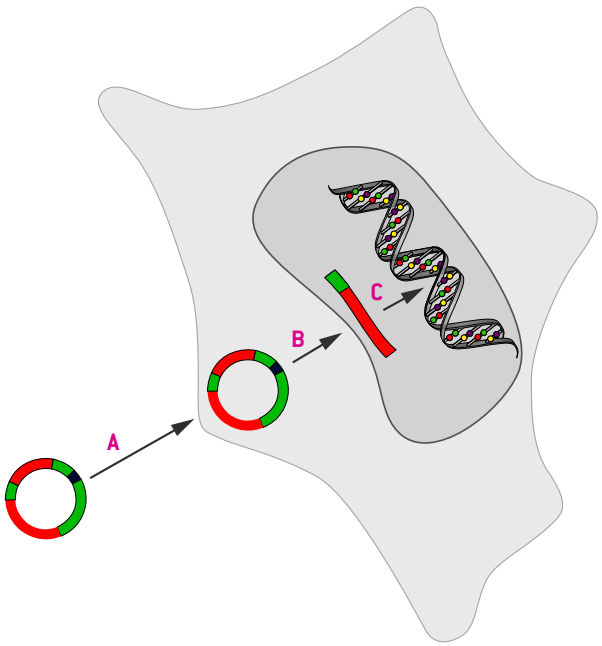


Figure 1. The general path of stable integration.

Table 1: Different Strategies for Stable Clone Generation

Culture System	Advantage	Application
Batch culture – polyclonal	Fast, useful for cells which do not grow in single cell culture	Overexpression, protein expression systems (e.g., for basic research)
Limiting dilution – monoclonal	Defined cell clones	Studies of gene function, protein production (e.g., for therapeutic applications)

In a batch culture system, a mixed population of drug resistant cells is selected on plates or in flasks and can be used directly for experimental analysis. During a limiting dilution procedure, cells are usually diluted and selected e.g., in a 96-well plate for outgrowth of cell clones or single colony growth. Subsequently, colonies can be picked and used to generate monoclonal cell lines. Tip: By using a combination of these two methods one can reduce the number of plates for screening plus costs and efforts. Starting with a batch selection prior to performing limiting dilution will pre-select and reduce the amount of cells which have to be plated in a limiting dilution.

Culture Conditions for Generation of Stable Cell Lines

As for transient transfection experiments, culture conditions (passage number, split rhythm, etc.) of your selected cell type are very important for the generation of stably-transfected cell lines. For optimal results, we recommend using the cell culture recommendations of the supplier for the respective cell type. In general, the cell line should be passaged two days before the experiment to promote good proliferation and cell physiology. Cell passage should not be higher than 30. Interference of higher passage numbers with integration efficiency is possible and may be cell-type dependent.

Depending on the scope of your experiment, cells can be cultivated as polyclonal batches or monoclonal single cell clones post transfection.

Transfection Method

Stable expression can be influenced by the transfection method used. The choice of transfection method determines which cell type can be targeted for stable integration. While biochemical transfection reagents can be used to transfer DNA into standard cell lines, efficient delivery of DNA into notoriously difficult-to-transfect suspension cell lines or even primary cells is only possible with viral methods or Nucleofection (Figure 2). Unfortunately, viral methods suffer from several limitations, such as time consuming production of vectors and safety concerns (Hacein-Bey-Abina *et al.*, 2003).

We recommend using the Nucleofector™ Technology (see page 233-240), for transfection of difficult-to-transfect cell lines.

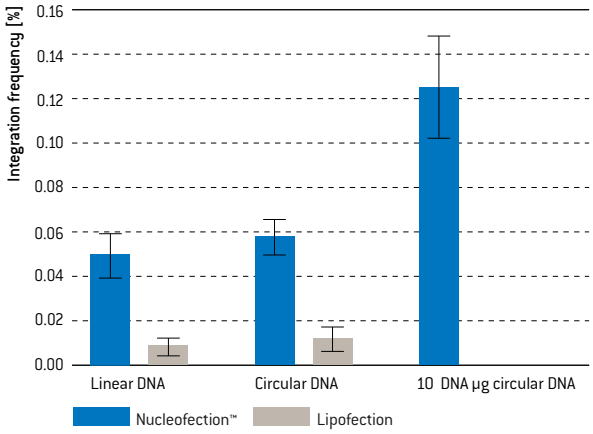
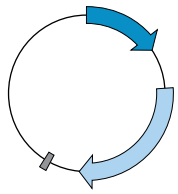
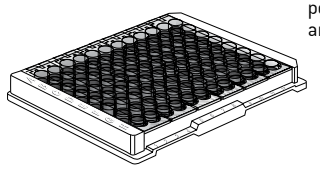
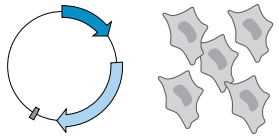
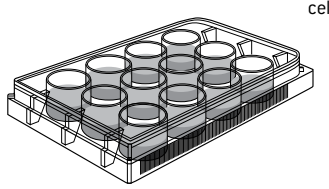
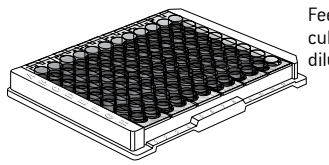
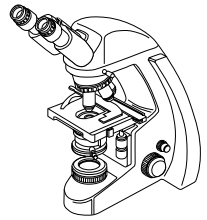


Figure 2. Higher integration rates in difficult-to-transfect cell lines using Nucleofection. Jurkat cells were transfected using either Nucleofection (2 µg DNA) or lipofection Reagent L (0.7 µg DNA) according to the respective manufacturer's instructions. 24 hours after transfection, cells were plated on a 96-well plate containing culture medium supplemented with G418 for selection of stably-transfected cells. 30 days after plating, cells were analyzed for clonal outgrowth (Integration frequency = number of resistant clones per number of living cells seeded). Due to toxic effects, lipofection with 10 µg circular DNA could not be performed.

Guideline for Generation of Stable Cell Lines

Continued

Experimental Outline

Expression Plasmid	Procedure Outline	Important Information
Step 1 Expression plasmid 	Design experiment and choose cell type, expression vector and transfection method.	Make sure that transfection method and expression vector are suitable for your cell type.
Step 2 	Determine appropriate cell number per well (only for limiting dilution) and G418 concentration.	Cells differ in their susceptibility to G418. The active concentration of stock G418 can vary from batch to batch.
Step 3 Expression plasmid + cells 	Transfect expression vector into cells.	Amount of expression vector per experiment is dependent on transfection method and cell type.
Step 4 	Plate transfected cells and cultivate cells in medium without G418.	Do not add G418 to culture medium immediately after transfection as this may drastically increase mortality.
Step 5 	Dilute cells into culture plates and start selection 24–48 hours post-transfection. Feed every 2–3 days (for batch culture) or 10 days (for limiting dilution) with selection medium.	Choose culture conditions (batch culture, limiting dilution) depending on your experimental design. Refreshed selection medium is important to avoid false positive cells.
Step 6 	Analyze stably transfected cells.	Make sure that the chosen assay is suitable for your application.

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Protocol for Batch Culture

Determination of G418 Concentration Using Batch Culture

Stably-transfected cells can be selected by the addition of drugs to the culture medium, if the expression plasmid carries a drug resistance gene. Here we describe the neomycin resistance system, which uses resistance to G418 as a selection marker. Cells differ in their susceptibility to G418, which may even vary with cell passage numbers. Cells that are cultured in serum-free media may require much lower G418 concentrations as compared to cells in media containing sera. The selection condition for your specific cell type needs to be established experimentally. Determine the minimum level of G418 to be added to the culture medium to prevent cell growth. Note that the active concentration of stock G418 can vary considerably from batch to batch. We therefore recommend testing G418 sensitivity for every new batch or to buy a large amount of one lot to standardize selection conditions.

1. Split cells into 12-well plates containing culture medium without G418 in plating densities according to cell supplier instructions.
2. The next day, aspirate growth medium and feed cells with medium containing increasing concentrations of G418 (e.g., titrate G418 in a range of 0.1 mg/mL to 1.5 mg/mL; in serum-free culture expand range down to 20 µg/mL).
3. Feed cells every 2–3 days with selection medium.
4. Check cell death after 7–14 days by light microscopy.
5. Choose the concentration which is 0.1 or 0.2 mg/mL above the one which shows complete cell death as the appropriate G418 concentration for selection. If the lowest concentration used shows complete cell death at day 7, the titration should be repeated with a lower concentration range.

Transfection

For transfection, please follow the respective manufacturer's instructions of your transfection system and transfect the expression plasmid, containing the gene of interest and the sequence for a drug resistance gene, into your cell type. After transfection, plate cells according to the instructions from the supplier of your transfection system on tissue culture plates. Usually 6-well plates are used for 10^6 adherent cells and 12-well plates for 10^6 suspension cells.

Important controls:
We suggest including a sample of untransfected cells as a negative control for selection. We also strongly recommend checking the transfection efficiency and integration frequency of your experiment with a GFP-control plasmid, containing a selection marker, e.g. pTurboGFP (Evrogen).

Cell Culture Post Transfection

Under selective conditions, resistant cells outgrow non-resistant cells, resulting in a polyclonal population of stably-expressing cells. This heterogeneously expressing population of resistant cells can then be used for experimental analysis.

1. After transfection, allow cells to grow and to express the protein for G418 resistance under non-selective conditions for at least 24 hours (for sensitive cells, G418 selection may begin after 48 hours).
2. Trypsinize adherent cells by standard procedures or use suspension cells directly for analysis. If possible, analyze for transfection efficiency 24–48 hours post-transfection on an aliquot of the positive control sample and your gene of interest (transient transfection control).
3. For the selection of stably expressing cells, cultivate cells in standard medium with supplements and the appropriate amount of G418, pre-tested for your cell type.
4. Plate cells on culture plates or flasks according to cell supplier instructions and incubate cells under standard conditions.
5. Feed and, if necessary, split cells until outgrowth of resistant cells.
6. Harvest cells of batch culture.

Usually, cells which have not integrated the resistance gene die during the first days of selection. Outgrowth of resistant cells can be observed normally after 2 weeks of selection. For some cells this may take up to 4 weeks. G418 is labile at 37°C, therefore it is recommended to change medium containing G418 every 2–3 days to compensate for loss of selection pressure. In some cases, it might be feasible to lower the G418 concentration after 1–2 weeks. Cells should be grown for at least 3 weeks under selection pressure to avoid contamination with non-resistant cells. Negative control wells (e.g., sample without expression plasmid) should be inspected by light microscopy and should not contain any signs of cell growth. Dependent on cell type and cell growth, selection can be extended up to 4–5 weeks or longer in total.

Guideline for Generation of Stable Cell Lines

Continued

Analysis of Batch Culture

Once you have obtained resistant cell batches or clones, expand the cells and assay for your gene of interest.

Protocol for Limiting Dilution

Determination of G418 Concentration and Plating Density

Stably-transfected cells can be selected by the addition of drugs to the culture medium, if the expression plasmid carries a drug resistance gene. Here we describe the neomycin resistance system, which uses resistance to G418 as a selection marker. Cells differ in their susceptibility to G418, which may even vary with cell passage numbers. Cells that are cultured in serum-free media may require much lower G418 concentrations as compared to cells in media containing sera. The selection condition for your specific cell type needs to be established experimentally. Determine the minimum level of G418 to be added to the culture medium to prevent cell growth. Note that the active concentration of stock G418 can vary considerably from batch to batch. We therefore recommend testing G418 sensitivity for every new batch or buying a large amount of one lot to standardize selection conditions. The final plating density after transfection depends on the culture conditions of the specific cell type and the G418 concentration. We therefore recommend combining the titration of G418 with the titration of cell numbers for determination of plating density in a matrix (Figure 3).

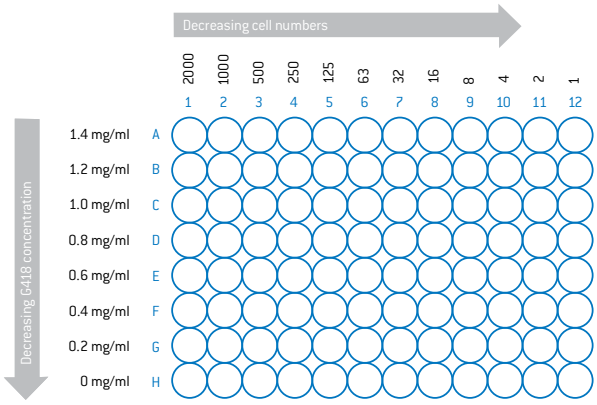

Figure 3. Matrix titration of G418 and titration of cell number for determination of plating density in one 96-well plate.

1. Pre-plate 100 µL medium in each well of the plate.
2. Add 100 µL of cell suspension containing 4000 cells per well to the first column (#1).
3. Carry over 100 µL to the next column after gentle up and down pipetting, thereby diluting in a ratio of 1:2. Repeat this procedure for each consecutive column.
4. After completing, discard 100 µL from the last column (#12). The first column should then contain about 2,000 cells, the last column less than one cell on average.
5. Add 100 µL of G418 containing medium (2.8 mg, G418 per mL) to the first row (A) for a final G418 concentration of 1.4 mg/mL.
6. Add G418 to the following rows in decreasing concentrations of G418 in steps of 0.2 mg/mL. For the last row (H) add medium without G418.
7. Incubate cells at standard conditions.
8. Analyze cell growth by microscope. In some cases, cell growth can also be observed by change of medium color.
9. If you observe cell growth (after >10 days) in the wells without G418 containing less than 4 cells, it is reasonable to assume that those cells can grow out starting as single cells.
10. Choose the G418 concentration which is just above the one which shows complete cell death as the appropriate G418 concentration for selection.

Certain cell types might need a critical number of neighboring cells to grow appropriately. If this is the case, limiting dilution experiments can only be done at higher cell concentrations, making it more difficult to obtain pure clones. In these cases, clones should be generated by plating an appropriate number of cells, selecting and diluting the resistant clones together with non-transfected cells of the same type as feeder cells. Other possibilities are culturing cells in 96-well plates with communicating channels, on soft-agar or methylcellulose.

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Guideline for Generation of Stable Cell Lines

Continued

Transfection

For transfection, please follow the respective manufacturer's instructions of your transfection system and transfect the expression plasmid containing the gene of interest and the sequence for a drug resistance gene into your cell type.

After transfection, plate cells according to the instructions from the supplier of your transfection system (e.g., on 96-well tissue culture well plates).

Important controls:
We suggest including a sample of untransfected cells as a negative control for selection. We also strongly recommend checking the transfection efficiency and integration frequency of your experiment with a GFP-control plasmid containing a selection marker, e.g. pTurboGFP (Evrogen).

Cell Culture Post-transfection

Single cell clones from adherent and suspension cell types can be generated by diluting cells in a 96-well plate or other methods that allow the outgrowth of isolated cell clones under selective pressure.

1. After transfection, allow cells to grow and to express the protein for G418 resistance under non-selective conditions for at least 24 hours (for sensitive cells, G418 selection may begin after 48 hours).
2. Trypsinize adherent cells by standard procedures or use suspension cells directly for analysis. If possible, analyze for transfection efficiency 24–48 hours post-transfection on an aliquot of the positive control sample and your gene of interest (transient transfection control).
3. Count living cells via trypan blue staining or other appropriate methods.
4. Use standard medium with supplements and the appropriate amount of G418 pretested for your cell type and plate cells in a 96-well plate with different cell numbers per well (e.g., 10, 100, 1000) in a volume of at least 100 µL per well. Depending on cell concentration determined before, conduct several serial dilution steps as applicable. It is important to thoroughly suspend cells before seeding, but avoid harsh treatment by frequent pipetting. Use the lower limit determined before as the minimum number of cells per well (for generation of single cell clones, choose the dilution which statistically yields between 5 and 20 clones per 96-well plate, thereby minimizing the probability of wells with more than one clone).
5. Incubate cells under standard conditions and feed cells after 10–14 days with fresh selection medium.
6. Cell clones can be analyzed or further expanded as soon as cells in the non-transfected control wells have completely died.
7. In order to help assure that selected cell populations represent clones from a single cell, another round of limiting dilution under selection is recommended. Usually, cells which have not integrated the resistance gene die during the first days of selection. Outgrowth of resistant cells can be observed normally after 2 weeks of selection. For some cells, this may take up to 4 weeks. G418 is labile at 37°C, therefore, it is recommended to add fresh medium containing G418 after 10–14 days to compensate for loss of selection pressure. In some cases, it might be feasible to lower the G418 concentration after 1–2 weeks. Cells should be grown for at least 3 weeks under selection pressure to avoid contamination with non-resistant cells. Negative control wells (e.g., sample without expression plasmid) should be inspected by light microscopy and should not contain any signs of cell growth. Dependent on cell type and cell growth, selection can be extended up to 4–5 weeks or longer in total.

Analysis of Stable Clones

Once you have identified resistant clones, expand the cells and assay for your gene of interest by using an appropriate analysis method (e.g., microscopy, flow cytometry, ELISA). For the analysis of the positive control cells, use fluorescence microscopy to screen the 96-well plate.

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Guideline for Generation of Stable Cell Lines

Continued

Table 2: Troubleshooting

Symptom	Suggestion
Transient transfection efficiency is low	Optimal cell density should be determined for each cell type. For adherent cells, the optimal confluency at the time of transfection is normally 60–80%. Higher, as well as substantially lower, cell densities may cause lower transfection efficiencies. Suspension cells must be in their logarithmic growth phase. Choose appropriate transfection method (e.g., Nucleofection for difficult-to-transfect cell lines).
Viability is low 24 hours post-transfection	Try lower DNA amounts when using cells known to be DNA sensitive. Check passage number, split rhythm and medium in cell supplier instructions for your cell type. Choose appropriate transfection method (e.g., Nucleofection for difficult-to-transfect cell lines).
Transfected cells do not grow in 96-well plates, even without G418	Re-titrate plating densitiy for optimal cell growth. Don't go below minimal cell number for single cell growth even without selection. Check passage number, split rhythm and medium in cell supplier instructions for your cell type. Use flat-bottomed plates for adherent cells and round-bottomed plates for suspension cells.
Number of resistant clones in 96-well plates is low after selection	Check transient transfection efficiency of your transfection method (e.g., using maxGFP™ Reporter Protein as a control). Try higher DNA amounts. Re-evaluate G418 amount for optimal cell growth in single cell cultures. Try lower G418 concentration. Re-check the optimal plating density in 96-well plates. If correct, increase cell numbers per well. Control passage number of cells and confluency of cells before transfection. Choose appropriate transfection method (e.g., Nucleofection for difficult-to-transfect cell lines).
After selection, too many resistant clones mixed with non-resistant cell clones in 96-well plates Clones are growing on negative control plate	Feed cells with fresh G418 selection medium at least 14 days after transfection. Use the same batch of G418 you used for initial G418 titration. Re-check the optimal plating density in 96-well plates. If correct, decrease cell numbers per well. Use a similar passage number (difference not more than 10) of cells for titration of G418 and for transfection and selection.
Clone does not grow out after selection	Wait 4–5 weeks before picking a resistant clone to obtain a sufficient number of cells for culture expansion. Use the same batch (and concentration) of G418 you used for initial G418 titration.
Clone is resistant but gene of interest does not show expression in several clones checked	Check expression of gene of interest in a transient expression assay, if possible. Try linearizing the plasmid before transfection, this prevents disruption of gene of interest during integration. Check the sequence of gene of interest for ATG and Stop-Codon.
Positive control provides high number of resistant clones, but gene of interest does not	Reverify correct insertion of gene of interest and the resistance marker into plasmid by sequencing or restriction digest. Check whether the expressed recombinant protein is toxic to the cells.

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Designing an RNAi Experiment Using Nucleofection

The Nucleofector™ Technology is well suited for the transfection of siRNA duplexes or shRNA vectors into both primary cells and difficult-to-transfect cell lines.

Choose siRNA

- **Select gene target(s)**
- **Select control siRNA**
  - Negative control siRNA – e.g., Thermo Scientific siGENOME Non-Targeting Control*
  - Positive control siRNA – e.g., siGENOME® GAPDH siRNA*

Choose Cell Type and Transfection Protocol

- **Select cell type(s) to maximize physiological relevance of results**
- **Find Optimized Nucleofection Protocols at www.lonza.com/cell-database**
- **Select transfection controls**
  - Untreated sample (no siRNA and transfection)
  - Mock-transfection (no siRNA, only transfection)

Confirm siRNA Delivery

- **Confirm siRNA delivery efficiency using:**
  - Fluorescently-labeled siRNA
    or
  - Fluorescent expression plasmid (e.g., pmaxGFP™ Vector)
    or
  - pmaxGFP™ Vector and maxGFP™ Reporter Protein siRNA
    or
  - siRNA targeting housekeeping gene

Choose Detection Assay

- **Select detection assay(s)**
  - mRNA – branched-DNA, RT-PCR
  - Protein – ELISA, Western, FACS analysis
  - Phenotype – viability, apoptosis

Optimize Target Knockdown

- **Determine optimal siRNA concentration**
  - 100 µL reaction → 0.2–200 pmol (2 n–2 µM)
  - 20 µL reaction → 0.04–40 pmol (2 nM–2 µM)

Adapt Assay Conditions

- **Optimize detection assay(s) conditions for specific system**
  - Determine optimal cell densities for linear detection range
  - Correlate results from multiple assays

Optimize Assay Conditions

- **Optimize Assay Conditions**
  - Perform detection time course or multiple assays
  - mRNA → 12–72 hours
  - Protein → 24–96 hours

Confirm Specificity of Silencing Event

- **Confirm gene knockdown results with different siRNA reagents**
  - If using Thermo Scientific siGENOME SMARTpool siRNA Reagents*, follow-up with Thermo Scientific ON-TARGET*plus* SMARTpool siRNA Reagents* targeting same genes
  - If using individual siGENOME® siRNA*, use multiple siRNAs targeting same genes
- **If possible, perform rescue experiments**

*Thermo Fisher Scientific, Dharmacon Products

Designing an RNAi Experiment Using Nucleofection

Continued

Establish/Verify Nucleofection Conditions with pmaxGFP™ Vector

Optimal Nucleofection Conditions for a particular cell type are identical whether you are transfecting DNA or RNA.

We recommend performing a preliminary experiment with pmaxGFP™ Vector (our positive control plasmid, included in every kit) in order to establish/verify the optimal Nucleofector™ Solution and Program for your cells. Once these conditions have been determined, they remain the same whether you are transfecting DNA or RNA (or both together).

Identify Appropriate Experimental Controls

To make sure that the conclusions drawn from siRNA experiments are accurate, it is necessary to include the appropriate experimental controls. We recommend including at least four types of experimental controls in every RNAi experiment. Parallel testing of multiple controls under several conditions can be easily performed using the 96-well Shuttle™ System.

- **Positive siRNA Control**
  This should be a validated siRNA pool or individual siRNA targeting a well-characterized housekeeping gene, such as cyclophilin B (also known as PPIB), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), or Lamin. A good positive control targeting a well-expressed but non-essential gene is useful for establishing experimental parameters without affecting cellular viability. It can also be used as a negative control that is not associated with any particular pathway under study (i.e., it fails to generate an observable phenotype in the assay being employed).

- **Negative siRNA Control**
  Negative siRNA controls are bioinformatically designed and validated to have no known target in the cell type of choice. These reagents are important for distinguishing sequence specific silencing from sequence-independent effects that are associated with the delivery of siRNA into the cell. Such sequence-independent effects can include toxicity resulting from the process of transfection in conjunction with nucleic acid delivery or hypersensitivity to introduction of double stranded RNA. Investigators are encouraged to test multiple candidates in their own experimental systems to empirically confirm that the negative controls do not result in any observable and unintended off-target effects. For that purpose, Thermo Fisher Scientific offers a comprehensive portfolio of multiple negative controls, including the ON-TARGET*plus*® Non-Targeting Controls, which have been confirmed by microarray analysis to have little to no off-target signature in HeLa cells.

- **Untreated Transfection Control**
  The untreated control sample is comprised of cells that have neither been treated with siRNA nor subjected to the transfection process. This control serves as an indicator of baseline cellular activity to which all other conditions can be compared.

- **Mock-treated Control**
  The mock-treated control sample is one in which the cells are subjected to the transfection procedure in the absence of siRNA. In the case of Nucleofection, the cells would be exposed to the Nucleofector™ Solution and subjected to the Nucleofection Procedure in the absence of siRNA. The analysis of mock-treated cells will indicate whether the transfection process results in cytotoxicity or other non-specific effects.

Fine-tune Specific siRNA Sequence/Concentrations

Using the same Nucleofection Conditions, simply substitute siRNA for the pmaxGFP™ Vector used in the preliminary experiment. We often include the pmaxGFP™ Vector (either in a separate parallel sample or co-transfected by Nucleofection with the siRNA) as an easy means of comparing relative transfection efficiencies between experiments or selecting transfected cells. The transfection efficiency using DNA is usually substantially lower as compared to siRNA. If you are interested in using fluorescently labeled oligonucleotides, please first read our additional notes at the end of this article or contact our Scientific Support Team for specific suggestions.

- **Selection of an Optimal siRNA Sequence**
  If you are using siRNA sequences which have not been previously characterized, we recommend investing a considerable amount of time in their selection. The majority of siRNA providers offer an oligonucleotide optimization service, however, it is still often necessary to test several gene-specific siRNA oligonucleotides in order to find one which efficiently downregulates your target gene.

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Table 1: Using Low siRNA Concentrations with Nucleofection

siRNA Concentration	Cell Type	Targets/Analysis Method	Knockdown	Reference
2 nM (0.2 pmol*)	COS-7 (monkey kidney fibroblast)	Bruton's tyrosine kinase / FACS	96%	5
7 nM (0.7 pmol*)	THP-1 (human monocytic leukaemia)	Interferon Regulatory Factor (IRF5) / RT-PCR	»strongly reduced«	6
1 nM (0.1 pmol*)	HUVEC (human umbilical vein endothelial cells)	Interferon Integrins 1 / 3 and Akt / Western Blot Migration	>90%	7

* Per cuvette (100 µL volume)

Determination of the Optimal Effective siRNA Concentration

When performing siRNA-mediated knockdown experiments, it is advisable to conduct a dose-response (concentration) analysis to determine the minimum siRNA concentration necessary for sufficient target knockdown on mRNA, protein or functional level. For Nucleofection, the optimal siRNA concentration can range from lower than 2 nM up to 2 µM, depending on multiple factors such as the cell type and the half-life of the mRNA and/or protein of the gene target. To determine the optimal concentration for your cell type and target, we suggest performing an initial titration of the siRNA concentration within the range of 2 nM – 2 µM (0.2–200 pmol in 100 µL; 0.04–40 pmol in 20 µL). Starting concentrations for a minimum titration would be 30 and 300 nM. If looking at concentrations, these values may seem higher than with lipid-based methods, but it is important to remember that Nucleofection occurs in a 5x to 25x lower volume (20 µL with Nucleocuvette™ Strips vs. 100 µL with 96-well lipofection; 100 µL single Nucleocuvette™ Vessels or aluminum cuvettes vs. 1–2.5 mL with 6-well lipofection).

The optimal effective siRNA concentration is dependent on the target and the cell type. Indeed, there are numerous publications in which Nucleofection of <50 nM siRNA has been observed to elicit knockdown of the desired genes (see Table 1).

Some customers have also reported satisfying results with concentrations higher than 1 µM, but it is important to keep a balance between efficient knockdown and minimizing off-target effects. Although keeping siRNAs <30nt avoids activating the protein kinase (PKR) and 2',5'-oligoadenylate synthetase pathways, siRNAs still elicit non-specific effects, including both stimulation and repression of non-target genes.[1]

Determine Optimal Analysis Time Point

As the stability and half-life of various mRNAs and their protein products varies, it is important to empirically determine the best time points for assessing target knockdown. For example, it has been documented that in mammalian cells, mRNA half-life can range from minutes to days[2] while the half-life of protein products can range from less than a few minutes to several days. Taking this into consideration, the experimental design should allow sufficient time for the siRNA to associate with RISC and deplete mRNA/protein concentrations to desired levels.

In general, the recommended time course ranges are 5–72 hours to deplete target mRNA and 24–96 hours to adequately knockdown target proteins and assess phenotypic outcomes.

Verification of siRNA Specificity

Keeping siRNA concentrations as low as possible helps to minimize non-specific effects, but it is also important to include appropriate controls in all experiments[3]. Monitoring gene knockdown at both the mRNA and protein levels verifies that the siRNA sequence is acting through the classical RNAi pathway, rather than as a microRNA (which, at least in part, inhibit translation of target mRNA, rather than targeting its destruction). A good way to enhance confidence in RNAi data is to demonstrate a similar effect with two or more siRNAs targeted to different sites in the RNA transcript of interest. The rescue experiment as ultimate control: As suggested in the *Nature Cell Biology* editorial[3], the control of choice for any RNAi experiment is rescue by expression of the target gene in a form refractory to the siRNA, usually achieved by utilizing one or more silent third codon point mutations or a deletion in the untranslated region within the targeted sequence. Translational effects can be avoided by using siRNAs targeted against 3'-untranslated regions. In practical terms, a rescue experiment means either co-transfecting the siRNA and a plasmid expressing the siRNA-resistant form of the target gene together, or using an shRNA-expression vector which co-expresses the siRNA-resistant target gene. Fortunately, the ability to transfect DNA and RNA using identical Nucleofection Conditions means that both of these types of experiments are quite straight forward and easy to perform using the Nucleofector™ Technology.

Designing an RNAi Experiment Using Nucleofection

Continued

Amount	Weight Molecular weight of a 21 bp siRNA ds-oligonucleotide: 21 × 660 g/mol = 13860 g/mol = 13.86 ng/pmol ≈ 14 ng/pmol	Concentration 100 µL Nucleocuvette™ Vessel	Concentration 20 µL Nucleocuvette™ Vessel
1 pmol	14 ng	1 pmol / 100 µL = 10 nmol/L = 10 nM	1 pmol / 20 µL = 50 nmol/L = 50 nM
5 pmol	69 ng	5 pmol / 100 µL = 50 nmol/L = 50 nM	5 pmol / 20 µL = 250 nmol/L = 250 nM
10 pmol	140 ng	10 pmol / 100 µL = 100 nmol/L = 100 nM	10 pmol / 20 µL = 500 nmol/L = 500 nM
20 pmol	277 ng	20 pmol / 100 µL = 200 nmol/L = 200 nM	20 pmol / 20 µL = 1000 nmol/L = 1 µM
50 pmol	690 ng	50 pmol / 100 µL = 500 nmol/L = 500 nM	50 pmol / 20 µL = 2500 nmol/L = 2.5 µM
100 pmol	1.4 µg	100 pmol / 100 µL = 1000 nmol/L = 1 µM	100 pmol / 20 µL = 5000 nmol/L = 5 µM

For individual calculation, also refer to our website www.lonza.com/sirna-calculator

Additional Notes

Measuring Transfection Efficiency Using fluorescently-labeled siRNA

Experiments with fluorescently-labeled siRNAs have shown transfection efficiencies of up to 99% in some cell types. Unless a confocal microscope or FACS is available, the use of fluorescently-labeled siRNA for initial setup experiments is not advisable, as many fluorescent labels fade quickly following Nucleofection. Likewise, the amount of fluorescently-labeled siRNA needed in order to adequately visualize fluorescent cells is often much higher than would be optimal for functional response, making this both an expensive and not highly informative experiment. Furthermore, microscopic analysis may lead to false positive results as a result of siRNA sticking to the membrane and not actually entering the cell. We suggest including a sample transfecting pmaxGFP™ Vector in parallel to (or in the same sample as) the siRNA in order to provide a general estimate of relative transfection efficiency. Nevertheless, take into account that the transfection efficiency of siRNA molecules is usually much higher. If you wish to use labeled siRNA for your experiments, please contact our Scientific Support Team to help your experiments run as smoothly as possible.

Enriching for Transfected Cells

One method of enriching for transfected cells is to co-transfect siRNA with a plasmid expressing a fluorescent reporter or surface marker, and then sorting for cells expressing the reporter. This approach has been used, for example, in Wu *et al.* (2005)[4]. Using shRNA-expressing vectors also allows you to use co-expressed fluorescent or antibiotic resistance markers to select for transfected cells (see below). However, transfection efficiencies for plasmid DNA are generally lower than those for siRNA duplexes.

Longterm RNAi Effects (siRNA duplexes vs. shRNA-Expressing Vectors)

Chemically synthesized siRNA duplexes offer a rapid means for determining the siRNA sequences that result in efficient knockdown of your target gene, but this downregulation is transient (generally persisting 2–5 days) and may not be sufficient when silencing targets with low turnover rates or in other applications where a longer duration of effect is required. Consequently, a number of different plasmid vectors that express siRNA, or shRNA (short hairpin RNA) are commercially available. In addition to enabling long-term expression of siRNA/shRNA, these vectors have the advantages that they can be grown, handled and stored as plasmid DNA, co-express fluorescent markers or antibiotic resistance genes (facilitating identification of transfected cells/selection of stably transfected cells) and can be engineered with inducible promoters to permit switching the knockdown phenotype on and off (such as pSuperior, OligoEngine). The ability of the Nucleofector™ Technology to transfect DNA into primary cells (and many cell lines which are difficult or impossible to transfect by other means) makes it possible to now use these vectors in virtually any cell type. Although do keep in mind that transfection efficiencies with siRNA oligonucleotides are generally higher than with plasmid DNA.

Stability of siRNA Duplexes in Nucleofector™ Solutions

The Nucleofector™ Solutions were tested for RNAse activity. Incubation of RNA in the solutions for two hours at 37°C did not affect RNA stability.

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This guideline provides a brief background on various genome editing tools and describes how to establish Lonza's Nucleofector™ Technology for genome editing applications in hard-to-transfect cell types, such as pluripotent stem cells.

1. Introduction to Genome Editing

The wealth of genomic sequence data now available to researchers has laid the foundation for a revolution in genetic modification technology. This technology, termed genome editing, provides the means by which heritable DNA alterations can be made at pre-determined specific sites in the genome.

In general, there are various options to modulate gene expression, be it on the DNA, RNA, or protein level. Many of these options only result in a transient modulation that might be sufficient or even advantageous for some approaches. However, prior to genome editing, a stable, heritable DNA modification was accomplished either by random integration of plasmids, transposons, or viruses or via homologous recombination. The latter method results in site-specific integration but is a very time-consuming and inefficient process. With the introduction of genome editing tools, site-specific stable modifications can now be performed easily. Zinc Finger Nucleases (ZFN) and Transcriptional Activator-like Effector Nucleases (TALEN) technologies were established over the last decade as useful tools for site-specific genomic modifications but, with the recent discovery of the CRISPR (clustered regularly interspaced short palindromic repeats) technology another potent alternative has emerged.

2. Applications

Genome editing technology has been applied in a wide variety of ways to effect genetic modifications in basic and applied research. Loss-of-function mouse knockout studies formerly accomplished by homologous recombination methods can now be performed rapidly and with greater efficiency due to the 10-100-fold increases in genetic modification rates with genome editing[1,2]. The fidelity and magnitude of gene expression decrease provided by genome editing has been shown to be superior to RNAi-based methods[3,4]. In addition RNAi-based methods only provide a transient knockdown. Several genome wide loss-of-function screens in tumor lines were recently carried out demonstrating the robustness of the technology[3,4]. Transgene insertions that site-specifically add a fluorescent protein, luciferase, or other reporter molecule have facilitated cell homing and lineage tracing studies that rely on preserving native cell function[5]. Cell models have also been created for monogenic diseases either by using patient-derived iPSCs or incorporating well-characterized mutations in iPSCs from normal individuals[6]. In addition to pre-clinical applications, therapeutically relevant cells have been modified with genome editing. For example, genome edited T-cells have been used in AIDS trials where the HIV-resistant form of the CCR5 gene replaced the normal allele[7].

3. Basics on Genome Editing Process and Tools

This chapter gives a brief introduction of the process and the main tools used. For more details please refer to the various reviews available (e.g. Gaj T *et.al.* 2013[8]).

For genome editing, engineered nucleases are used to delete, insert, or replace a gene at a targeted genomic location. Such engineered nucleases are typically comprised of two elements: an endonuclease DNA cleavage module, and a sequence-specific DNA binding domain.

The nuclease cleaves double-stranded DNA creating a double-strand break (DSB) (Figure 1). The DSB induces the cellular DNA repair process. There are two types of repair processes that can occur. Without a homologous donor fragment available – be it the corresponding allele or an external donor DNA – the broken ends will be re-joined. This process is called non-homologous end joining (NHEJ) and is often accompanied by a mutation that may cause a deletion of a functional element of the gene.

If a partially homologous donor sequence is present, e.g. the genomic allele or foreign donor DNA, an insertion or replacement of a gene can take place via homology-dependent repair (HDR). The frequency of NHEJ versus HDR depends on the individual experimental setting, e.g. the cell-type and the donor amount.

The combination of such nucleases with a sequence-specific DNA binding domain that can be customized to recognize virtually any sequence facilitates these repair processes in a targeted manner. The predominant DNA binding domains used in genome editing are zinc finger (ZF) proteins, transcriptional activator-like effector (TALE) proteins or CRISPR-guideRNAs (gRNA)

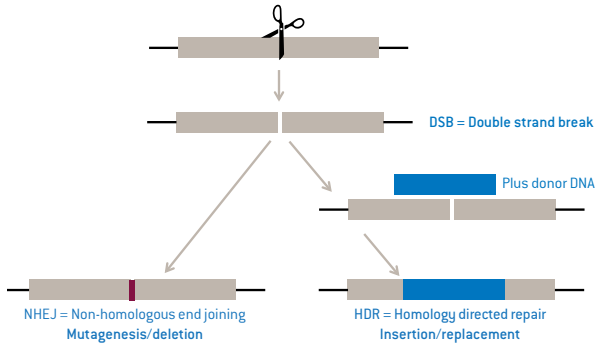

Figure 1. Cellular repair processes following the nuclease-induced double-strand break (simplified scheme).

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3.1 Zinc Finger Nucleases (ZFN)

Zinc finger (ZF) proteins are the most abundant and versatile DNA binding motif in nature[9]. An individual zinc finger domain binds 3 DNA base pairs. Because of their modular structure, they provide an ideal framework for designing an artificial sequence-specific binding molecule. This can be fused to an endonuclease which together mediate sequence-specific cleavage. Since its first proof-of-principle in 1996 by Kim *et al.*[10], ZFN-based genome editing technology has further evolved. The current generation of ZFNs utilizes 5 to 6 ZF domains, which recognize a genomic DNA stretch of 15-18 bp and are fused to Fok I nuclease. Two such zinc finger-nuclease fusion proteins work in combination to bind the sense and antisense strand of the targeted DNA sequence (Figure 2). Once both partners have bound, the Fok1 nuclease can form an active dimer and induce the double-strand break that leads to subsequent cellular repair processes. Since ZFNs target a total of 30-36 bp they provide a highly specific genome editing tool.

3.2 Transcriptional Activator-Like Effector Nucleases (TALEN)

In 2009 transcriptional activator-like effectors (TALEs) were discovered to provide a simpler, modular DNA recognition code[11, 12]. TALEs are naturally occurring proteins from the plant pathogenic bacteria genus Xanthomonas, and contain DNA-binding repeats, each recognizing a single base pair. Compared to the triplet-based DNA binding of zinc fingers, this single base recognition mode of TALE–DNA binding repeats enables greater flexibility in design but also holds some cloning challenges. Thus, except for the binding mode, the principle of targeting is very similar. Again sequence-specific, engineered TALEs are typically fused to Fok1 nuclease* to build the TALE-nuclease fusion (TALEN). As with ZFNs, a pair of TALENs must be generated for each target with each monomer binding 13 or more base pairs on the sense or antisense strand of the targeted DNA (Figure 2).

*Also other effector enzyme combinations might be used.

3.3 CRISPR/Cas9 System

Clustered regulatory interspaced short palindromic repeats (CRISPRs), discovered in 1987 in *E. coli*, were recently shown to provide an even simpler genome editing tool[13, 14, 15, 16]. The CRISPR pathway is part of the bacterial immune system to defend against invading viruses. This system has been adapted for use in eukaryotic cells. The specificity is driven by a so-called "guide RNA", which typically binds to a complementary stretch of 18-20 base pairs in the targeted DNA (Figure 2) and has some additional sequence motifs that help in forming a complex with the Cas9 nuclease (CRISPR-associated nuclease). For successful binding of Cas9, the genomic target sequence must also contain a correct Protospacer Adjacent Motif (PAM) sequence immediately following the target sequence. The PAM is an NGG motif adjacent to the binding site. In contrast to ZFNs and TALENs, for CRISPR-based genome editing the DNA binding domain and the nuclease are not fused, since the DNA binding part is an RNA and not a protein. This feature makes it much easier to design a new guide RNA addressing a new target and also allows for multiplexed targeting[16].

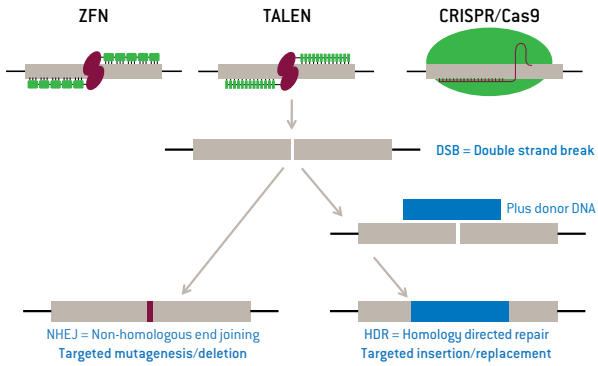

Figure 2. Sequence-specific induction of a double-strand break and subsequent repair processes (simplified scheme).

3.4 Comparison of ZFN, TALEN and CRISPR

Table 1 summarizes the main features of the three different genome editing tools. Briefly, ZFNs and TALENs require the generation of fusion proteins, thus making it more laborious to create a new engineered nuclease for another target site. For the CRISPR system only a new guide RNA needs to be generated to target another sequence. In addition, with CRISPR multiple targeting can be performed by combining the Cas9 nuclease with several guide RNAs.

On the other hand, currently ZFNs and TALENs are more specific than CRISPR and thus carry a lower risk for off-target effects. This is primarily due to their targeting of longer DNA stretches and the requirement for two partner molecules to form the final active nuclease dimer. To overcome this liability, some researchers have mutated the CRISPR Cas9 nuclease to a "nickase" which can then be used in conjunction with paired sense and antisense gRNAs thus providing enhanced specificity[17].

Most importantly, with the universal recognition of the potential of CRIPSR as a cutting edge technology much research is taking place to optimize the tool to suit specific applications.

Table 1. Brief Comparison of Genome Editing Tools

	ZFN	TALEN	CRISPR
Nuclease	Fok 1	Fok 1	Cas9
DNA binding via	ZF protein	TALE protein	GuideRNA (gRNA)
Type	Fusion protein – High effort to modify for new targeting site	Fusion protein – High effort to modify for new targeting site	Protein + RNA – Easy to modify Multiple targeting possible
Binding site	2 sites (15 or 18 bp each) – High specificity – Low risk for off-target effects	2 sites (≥ 13 bp each) – High specificity – Low risk for off-target effects	1 site (18-20 bp + 3bp NGG) – Lower specificity – Higher risk for off-target effects

3.5 Co-transfection

One feature that is common to all three tools is the need to co-transfer several substrates (plasmids, mRNAs, or oligonucleotides) into the cell type of interest for successful modification of genomic DNA (Figure 3). Co-transfection can be challenging, especially when it comes to hard-to-transfect cell types such as primary T cells, human embryonic stem cells (hESC) or induced pluripotent stem cells (iPSCs). This challenge is overcome by Lonza's non-viral Nucleofector™ Technology, (see chapter 5), which has been shown to work as a reliable and efficient method for transferring the required DNA-, RNA-, or even protein-based components into various cell lines, primary cells, and stem cells. It has proven to work with any of the genome editing technologies described above (Table 5).

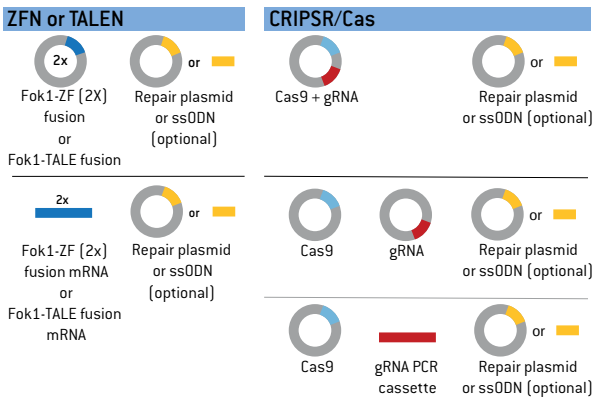

Figure 3. Possible co-transfection scenarios for ZFN, TALEN or CRIPSR/Cas9. The scheme shows some substrate type combinations (plasmids, mRNAs, or oligonucleotides) that have been described in the literature. However, additional scenarios may apply, e.g. transfection of proteins (see 4.4).

4. Using Nucleofector™ Technology for Genome Editing

4.1 Establish/Verify Nucleofection Conditions with pmaxGFP™ Vector

Lonza offers ready-to-use Optimized Protocols for a broad range of cell types (www.lonza.com/protocols) including hard-to-transfect cell lines and primary cells. Before performing a genome editing experiment we highly recommend to transfect our pmaxGFP™ Positive Control Vector to verify that the optimal conditions we identified also work well in the user-specific setting.

In case no ready-to-use protocol is available for a certain cell type, one can easily determine the optimal Nucleofection conditions using the pmaxGFP™ Vector by following the respective optimization protocol for a certain cell group or our general optimization protocols for primary cells or cell lines. For embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), for example, we recommend using our "Basic Stem Cell Protocol", since each ESC or iPSC clone may require slightly different transfection conditions. Once the optimal conditions have been determined, they remain the same whether DNA- or RNA-based substrates (or both together) are transfected.

4.2 Determination of Optimal Substrate Amounts

For successful genome editing it is important to determine the optimal substrate amounts. Tables 2 – 4 provide some example ranges for the different genome editing tools derived from published data. The ranges are given in amount per microliter to account for the different Nucleofection formats available (20 and 100 µL).

Table 2. Substrate Ranges Published for ZFN

Substrate	Range (per µL Nucleofection volume*)
ZFN plasmid (each)	0.01 – 0.05 µg/µL each
ZFN mRNA (each)	0.02 – 0.2 µg/µL each
Donor plasmid	0.04 – 0.2 µg/µL

*Note: Depending on the Nucleofection volume, ranges have to be multiplied by 20 or 100.

Table 3. Substrate Ranges Published for TALEN

Substrate	Range (per µL Nucleofection Volume*)
TALEN plasmid (each)	0.01 – 0.1 µg/µL each
Donor plasmid	0.05 – 0.2 µg/µL
Donor dsDNA (lin)	0.1 µg/µL
Donor ssODN	10 µM

*Note: Depending on the Nucleofection volume, ranges have to be multiplied

by 20 or 100.

Table 4. Substrate Ranges Published for CRISPR/Cas9

Substrate	Range (per µL Nucleofection Volume*)
Cas9/gRNA plasmid	0.025 µg/µL
Cas9 plasmid	0.02-0.05 µg/µL
gRNA plasmid	0.02-0.05 µg/µL
gRNA PCR Cassette	0.5 ng/µL
Donor dsDNA (lin)	0.02-0.1 µg/µL
Donor ssODN	0.5-10 µM
Cas9/gRNA ribonucleoprotein	See references 23 and 24

*Note: Depending on the Nucleofection volume, ranges have to be multiplied by 20 or 100.

4.3 Transfection of mRNA

Due to its shorter half-life the use of mRNA instead of plasmids might be beneficial when aiming to minimize the presence time of the nuclease and avoid multiple events. mRNA may also provide higher integration frequencies[18, 19].

When working with mRNA, the same protocol and program can be used that is optimal for the transfection of DNA into the respective cell type. However, there are a few additional things that should be considered:

- The mRNA should be capped and poly-adenylated
- As with plasmids the optimal mRNA amount has to be titrated, but it might be higher than for plasmid DNA (Table 2)
- If higher amounts are required, the total volume added to the transfection reaction should not exceed 10% of the total sample volume
- When collecting the cells for the transfection experiment you may want to include an additional wash step with PBS to get rid of serum-derived RNAses[19]
- Keep mRNA on ice prior to addition to the sample
- To avoid any degradation, e.g. due to prolonged contact with cells, the mRNA might be transferred directly into the empty cuvettes before adding the cell-solution mix on top and transfection should be performed immediately

4.4 Transfection of Protein

The Nucleofector™ Technology is also suited to transfect peptides[20,21,22] and proteins. As a starting condition we would recommend using the established optimal conditions for nucleic acids, but some program fine tuning might be required. Kim *et al.* (2014)[23] recently reported the transfection of Cas9-gRNA ribonucleoprotein using the 4D-Nucleofector™ System. They transfected K562, BJ or H9 cells with Cas9 protein premixed with in vitro transcribed gRNA. A similar approach was used by another research group who transfected Cas9-gRNA ribonucleoprotein into HEK293T cells, primary neonatal fibroblasts and H9 cells[24]. For protein ranges used please refer to the publications.

4.5 Factors Influencing Genome Editing Results

Besides the transfection efficiency, there are various factors that may influence the outcome of a genome editing experiment. For example, the integration frequency differs depending on the cell type selected[18]. In addition, as with any other substrate transfected, the quality of the genome editing tool used can have a major impact on the editing results. Tools from various non-commercial and commercial sources have been successfully tested in combination with the Nucleofector™ Technology (see Table 5).

When aiming for insertions via HDR, either double-stranded DNA or single-stranded oligonucleotides (ssODN) can been used as repair template. The latter provides an effective method for introducing single mutations and a simple format for screening approaches[18, 25].

5. Post Nucleofection – Selection and Expansion

Clonal selection can be started between 24 h and 7 days post transfection. The optimal time point has to be determined depending on the individual experimental setting.

One option to increase the number of clones is transfecting a vector that co-expresses a fluorescent protein, which would allow enrichment of transfected cells by FACS sorting.

For cells that do not like to be grown as single cells (e.g. ESCs or iPSCs) FACS sorting might also be an alternative to the limiting dilution process.

6. Analysis of Editing Events

Genome editing events can be analyzed by various means. Typically used methods comprise one or more of the following: PCR or RT-PCR, sequencing (e.g. deep sequencing, next generation sequencing), Southern blot, Northern Blot or mutation frequency assays (mismatch assays like e.g. Cel1 assay, T7 endonuclease I assay, SURVEYOR™ Nuclease Assay, or RFLP analysis) or Western blot (to analyze protein knockout). For iPSCs, Yang *et al.* (2014)[25] have developed a robust and user-friendly system (genome editing assessment system) using next-generation sequencing to screen for both HDR and NHEJ events.

7. Summary

The Nucleofector™ Technology is a very versatile method for transfection of multiple substrates in hard-to-transfect cell types. Here we provided some general recommendation about important factors to consider when using the technology for ZFN-, TALEN- or CRISPR-mediated genome editing. For more specific recommendations on a certain cell-tool combination you may refer to the respective publication (see Table 5). For example, Ran *et al* (2013)[26] gives comprehensive background information about CRISPR technology and provides a detailed protocol how to use Lonza's 4D-Nucleofector™ X Unit for CRISPR-based genome editing in HUES9 (a human stem cell line) and HEK293 cells. It also includes protocols for functional analyses, tips for minimizing off-target effects and FAQs. You may also contact our Scientific Support Teams for any specific guidance.

www.lonza.com/researchsupport

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Genome Editing Using Nucleofector™ Technology

Continued

Table 5. Selected publications for genome editing using the Nucleofector™ Technology

Tool	Authors	Citation	Year	Nucleofector™ Platform	Cell type
ZFN	Chen F *et al.*	Nat Meth 8(9):753-5	2011	Nucleofector™ II/2b Device	K562, HCT116, U2OS, HEK293, HepG2 and MCF7
	Fung H *et al.*	PLoS ONE 6(5):e20514	2011	Nucleofector™ II/2b Device	hESC
	Genovese P *et al.*	Nature 510:235ff	2014	4D-Nucleofector™ System	hCD34
	Hansen K *et al.*	J Vis Exp (64):e3304	2012	Nucleofector™ II/2b Device	K562
	Liu X *et al.*	PLoS ONE 7(5):e37071	2012	Nucleofector™ II/2b Device	hES
	Ou W *et al.*	PLoS ONE 8(11):e81131	2013	Nucleofector™ II/2b Device	iPSC
	Qu X *et al.*	Nucleic Acids Res 41:7771-7782	2013	Nucleofector™ II/2b Device	HIV-infected PBL and CD4 T cells
	Piganeau M *et al.*	Genome Res 23:1182-1193	2013	Nucleofector™ II/2b Device	hESC and Jurkat cells
	Richter S *et al.*	PLoS ONE 8(6):e65267	2013	Nucleofector™ II/2b Device	HTC116 and H460
	Robbez-Masson LJ *et al.*	PLoS ONE 8(11):e78839	2013	Nucleofector™ II/2b Device	MCF7
	Samsonov A *et al.*	PLoS ONE 8(7):e68391	2013	Nucleofector™ II/2b Device	A549
	Schjoldager K	PNAS 109:9893-9898	2012	n.d.	HepG2
	Torikai H *et al.*	Blood 119(24):5697-705	2012	Nucleofector™ II/2b Device	Human T cells
	Toscano MG *et al.*	Dis Model Mech 6:544–554	2013	Nucleofector™ II/2b Device	K562
	Wang J *et al.*	Genome Res 22:1316-1326	2012	Nucleofector™ II/2b Device and 96-well Shuttle™ Add-On	K562
	Zou J *et al.*	Blood 117:5561-5572	2011	Nucleofector™ II/2b Device	iPSC
	Zou J *et al.*	Blood 118:4599-4608	2011	Nucleofector™ II/2b Device	iPSC
	Yan W *et al.*	Scientific Rep 3:2376	2013	Nucleofector™ II/2b Device 4D-Nucleofector™ System	iPSC
TALEN	Piganeau M *et al.*	Genome Res 23:1182-1193	2013	Nucleofector™ II/2b Device	hESC and Jurkat cells
	Zhu F *et al.*	Nucleic Acids Res 10.1093/nar/gkt1290	2014	4D-Nucleofector™ System	iPSC and H9 hESC
	Yan W *et al.*	Scientific Rep 3:2376	2013	Nucleofector™ II/2b Device 4D-Nucleofector™ System	iPSC
	Yang L *et al.*	Nucleic Acids Res 41:9049-9061	2013	4D-Nucleofector™ System	iPSC
	Mussolino C *et al.*	Nucleic Acids Res 42(10):6762–6773	2014	Nucleofector™ II/2b Device	human newborn foreskin fibroblasts, K562
CRIPSR	Fu Y *et al.*	Nat Biotechnol 31(9):822–826	2013	4D-Nucleofector™ System	U2OS, K562
	Kim S *et al.*	Genome Res 24:1012–1019	2014	4D-Nucleofector™ System	K562, BJ fibroblasts
	Lin S *et al.*	eLife 3:e04766	2014	96-well Shuttle™ Add-On	HEK293T, human primary neonatal fibroblast and H9 hESC
	Petit CS *et al.*	J Cell Biol 202:1107-1122	2013	Nucleofector™ II/2b Device	HeLa
	Ran FA *et al.*	Cell 154:1380–1389	2013	4D-Nucleofector™ System	HUES62
	Ran FA *et al.**	Nat Prot 8(11):2281–2308	2013	4D-Nucleofector™ System	HUES9 and HEK293
	Yang L *et al.*	Nucleic Acids Res 41:9049-9061	2013	4D-Nucleofector™ System	iPSC

For more publications please refer to **www.lonza.com/citations.**

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Cell Culture Technical Information

We offer an extensive line of cell culture media and reagents backed by years of experience and innovation. Through our own internal efforts as well as work with exceptional collaborators, we are able to provide ongoing technical support in the form of protocols, detailed product information, and troubleshooting tips for our broad range of media and reagents. In this section we address many of the commonly asked questions relating to cell culture techniques by providing instructions and tips for adapting cultures to serum-free medium, cryopreservation and reconstitution, preparing powdered media, and more. Our Scientific Support Team is prepared to assist you with many other technical questions and concerns related to cell culture and media.

Adaptation of Cell Cultures to Serum-free Medium

The conversion of a particular cell or cell line from growth in serum-containing medium to serum-free medium is achieved through the weaning process. However, weaning is not required for all cell types. Rapid conversion of a cell population to serum-free conditions can be achieved by pelleting the cells and resuspending them in the serum-free medium. While this may be successful for some types of cells, a gradual conversion is more likely to yield the desired result.

Weaning is actually a process by which a subpopulation of cells that can proliferate in the absence of serum is selected. The degree of difficulty in selecting these cells is a function of the physical and nutritional requirements of the cells and the complexity of the serum-free formulation. Conversion of cells to growth in UltraCULTURE™ Serum-free Medium can be relatively simple because it is a complex formula. Other formulations may contain reduced amounts of protein (i.e., UltraCHO™ Medium and UltraDOMA™ Medium) or be entirely devoid of proteins and peptides (i.e., UltraDOMA-PF™ Medium). In practice, these formulations require slightly more attention during the weaning process. However, the benefits of a low protein serum-free growth environment and subsequent reduction in downstream processing procedures more than offset the extra time spent in the weaning process.

Maintenance of cellular function is an aspect of the weaning process that must be monitored. One needs to ensure that the subpopulation selected exhibits the same characteristics with respect to cellular function as the population that was cultivated in the presence of serum. These functions are diverse and may include receptor expression, viral susceptibility, monoclonal antibody production, and recombinant gene expression. In many cases, an increase in product yield has been noted when cells are converted to a serum-free environment. However, each investigator should monitor the cellular function of interest to their application during the weaning process.

We recommend two protocols for the conversion of cell populations to a serum-free environment. These protocols may be used for mammalian and invertebrate cell types. The first protocol may be used with attachment independent cells or cells that are loosely adherent and do not require trypsinization. It involves the gradual dilution of the serum-containing medium with serum-free medium. The second protocol may be used with both attachment dependent and independent cell types and begins with the serum-free medium supplemented with serum. A gradual reduction in the serum concentration is performed at each subculture until serum-free growth is achieved. This latter protocol has the added advantage of establishing the limit of serum concentration for the cell type. Some cells (especially transfected lines) require small amounts of serum (i.e., 0.1–0.5% v/v). This method allows the investigator to titrate the serum to the lower limit.

The two weaning protocols are presented on the following page. They represent our recommended procedures, however, each investigator may choose to make modifcations that better suit their particular application. In our experience, the minimum cell density maintained during the conversion process has a major effect on the outcome. We recommend that the cells be maintained above 3.0×10^5/mL for attachment independent and above 30% confluency for attachment dependent cells.

Protocols for Weaning Cell Cultures

Protocol #1: Medium Replacement – for Adherent Independent Cells (suspensions)

Approximate time required: 2 weeks – 6 weeks

Culture conditions:
- Mammalian cells: 95% air, 5% CO_2, 35°C–37°C
- Invertebrate cells: air, 25°C–27°C

1. Begin with cultures at maximum cell density.
2. NOTE: Attachment dependent cells that are exposed to trypsin during subculturing should be converted to serum-free growth using Protocol #2.
3. Split cells 1:2 using serum-free medium as the diluent.
4. Incubate cells until the maximum cell density is achieved.
5. Split cells 1:5 or to 3.0×10^5 cells/mL for attachment independent cells or 30% confluency for attachment dependent cells using serum-free medium as the diluent.
6. Incubate cells until the maximum cell density is achieved.
7. If the cell viability is >85% at this point, and the generation time is similar to that observed with serum-containing medium, the culture may be maintained in serum-free medium using a similar split schedule as originally optimized for serum-containing medium.
8. If the cells exhibit slow growth or low viability, maintain the split ratio at 1:2 or 1:5 for 3 successive splits. The minimum cell density should be above 3.0×10^5 cells/mL or 30% confluency during this period.
9. Gradually increase the split ratio to obtain a maximum value for the cell type being used.

NOTE: Some cells may require a small amount of serum for growth. If the cells have not adapted to serum-free cultivation using the above protocol, add 0.1%–0.5% serum to the culture or contact Scientific Support.

Protocol #2: Serum Dilution – for Adherent Dependent Cells

Approximate time required: 2 weeks – 6 weeks

Culture conditions:
- Mammalian cells: 95% air, 5% CO_2, 35°C–37°C
- Invertebrate cells: air, 25°C–27°C

1. Begin with cultures at maximum cell density.
2. Trypsinize attachment dependent cultures and transfer to an appropriately sized centrifuge tube. Attachment independent cells may be transferred directly to the centrifuge tube.
3. Sediment the cells by centrifugation at 350 × g for 5 minutes.
4. Resuspend the cells in serum-free medium containing 5% serum (v/v).
5. Adjust the cell concentration using the serum supplemented serum-free medium to a maximum of 3.0×10^5 cells/mL for attachment independent cells or a density to achieve not less than 30% confluency for attachment dependent cells.
6. Plant the cells and incubate until a maximum cell density is achieved.
7. Repeat steps 2–6 using a lower concentration of serum at each split. We recommend beginning at 5% serum and lowering to 2%, 1%, 0.5%, and finally 0.1% prior to eliminating serum from the culture.

NOTE: If the culture viability drops below 80% or if the generation time increases markedly following a decrease in the serum concentration, increase the serum level to the previous value and maintain the cells for 2 split cycles before lowering the level of serum again. It may be necessary to institute a more gradual decline in serum concentration with these cells. Some cell types may require a small amount of serum for growth. If the cells have not adapted to serum-free cultivation using the protocol described above, add 0.1–0.5% serum to the culture or contact Scientific Support.

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Basic Procedure for Cryopreservation and Reconstitution of Cultured Cells

NOTE: Not applicable to Clonetics™ and Poietics™ Primary Human or Animal Cells.

Cryopreservation

1. Select a flask of cells at or near confluency.
2. Cells should first be removed by trypsinization.
3. Adjust the cell concentration to between 2 × 10^6 and 8 × 10^6 cells/mL with EMEM (Cat. No. 12-136) containing 20% Fetal Bovine Serum. Centrifugation may be necessary.
4. Add the above cell suspension to an equal volume of cold (+4°C) Cryoprotective Freezing Medium (Cat. No. 12-132A).
5. Mix continuously to ensure homogeneity.
6. Dispense either 4 mL of the cells suspended in Cryoprotective Freezing Medium into 5 mL glass ampoules, or 1 mL into plastic screw cap vials suitable for freezing in the liquid or vapor phase of liquid nitrogen.
7. Cells are now ready for the freezing cycle. Cells should not be allowed to remain in the Cryoprotective Freezing Medium for more than 1 hour before freezing.
8. The temperature of the contents in the ampoule must then be lowered at a rate of 0.5°C–2°C/minute throughout the range of +4°C to -30°C.
9. After the temperature has reached -30°C, the rate of the temperature drop to -70°C (which is the warmest temperature at which cells can be stored) can be done very quickly. An automatic programmable freezer system is the most reliable means of obtaining controlled rate freezing.
10. Storage of the ampoules or vials must be at -70°C or colder. A storage temperature of -196°C (liquid nitrogen) is best. It is essential that the temperature of the contents of the ampoule be -70°C or colder at all times until reconstitution. For prolonged or indefinite storage, the use of liquid nitrogen is strongly recommended. Storage in dry ice or a mechanical freezer (-70°C) should be limited to less than3 months.

Ampoule Handling Recommendations

Receipt of Ampoule

Upon receipt, transfer the ampoule(s) from the shipping container to a -70°C freezer or a vapor phase nitrogen tank. For long-term storage (over 3 months), a vapor phase nitrogen tank is preferable to prevent significant loss of viability. Immersion of screw cap ampoules in liquid nitrogen is not recommended.

To Use

⚠ Caution: Wear protective facemask and clothing as ampoule explosions can occur.

1. Remove an ampoule from the freezer and place it into a 37°C waterbath. Do not submerge the ampoule or allow water to get under the cap.
2. After thawing, disinfect the ampoule with 70% isopropanol, then open aseptically in a laminar flow safety cabinet. Dilute the contents 1:10 in the appropriate growth medium.
3. Determine the viability and cell concentration of the thawed cells by using the Trypan Blue exclusion cell counting method.
4. Adjust the cell concentration as desired for seeding culture vessels.
5. Twenty-four hours after cells have been seeded, remove the medium and re-feed with the appropriate growth medium. This will remove the cryopreservative, if the alternative method described below is not used.
6. As an alternative, the cryopreservative may be removed prior to cell viability determination by centrifugation. This is done by centrifuging the resuspended cells at low speed for 10 minutes (200 × g). The supernatant is removed and the cells are resuspended in the appropriate growth medium.

Reference
Freshney, R.I. (2000) Culture of Animal Cells: A Manual of Basic Technique, 4th edition, Wiley-Liss, Inc., New York, pp. 297–308.

Determination of Cell Numbers

Counting cells by use of a hemacytometer is a convenient and practical method of determining cell numbers in suspension culture or from dispersed monolayer cultures. The hemacytometer consists of two chambers, each of which is divided into nine 1.0 mm squares. A cover glass is supported 0.1 mm over these squares so that the total volume over each square is 1.0 mm × 0.1 mm or 0.1 mm3, or 10^{-4} cm3. Since 1 cm3 is approximately equivalent to 1 mL, the cell concentration per mL will be the average count per square × 10^4.

Hemacytometer Counts are Subject to the Following Sources of Error:

- Unequal cell distribution in the sample.
- Improper filling of chambers.
- Failure to adopt a convention for counting cells in contact with boundary lines or with each other.
- Statistical error.

With careful attention to detail, the overall error can be reduced to about 15%. It is assumed that the total volume in the chamber represents a random sample. This will not be a valid assumption unless the suspension consists of individual separated cells. Cell distribution in the hemacytometer chamber depends on the particle number, not particle mass. Thus, cell clumps will distribute the same as single cells and can distort the final result. Unless 90% or more of the cells are free from contact with other cells, the count should be repeated with a new sample. Cells that are difficult to obtain in uniform suspensions, or in which extensive clumping cannot be avoided, may be counted by separating nuclei. This method is more time-consuming than direct counting and is subject to additional error if the population contains multinucleate cells. A sample will not be representative if the cells are permitted to settle before a sample is taken. Always mix the cell suspension thoroughly before sampling.

With a 10X objective and a 10X ocular, one square (1 mm^2) will approximately fill the microscope field (the circle on the representation of a hemacytometer grid). The cell suspension should be diluted so that each such square has between 20 and 50 cells (2–5 × 10^5 cells/mL). A total of 300 to 400 cells should be counted since the counting error is approximated by the square root of the total count. A common convention is to count cells that touch the middle line (of the triple lines) to the left and top of the square, but not to count cells similarly located to the right and bottom (see diagram).

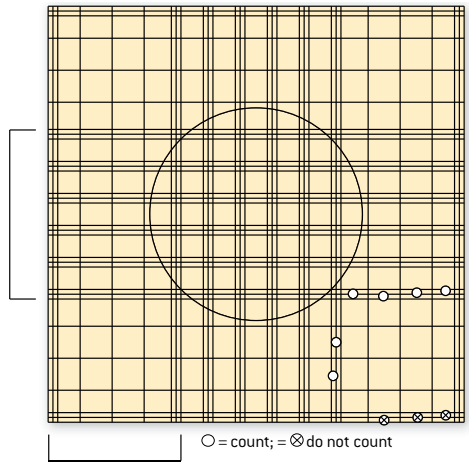

Diagram of a hemacytometer, improved Neubauer ruling, 0.1 mm deep Brackets indicate 1 mm^2 squares. Circle is the approximate area covered at 100X magnification.

In order to fill the hemacytometer chamber properly by capillary action, the cover slip, chamber, and the pipette used to fill the chamber must be scrupulously clean. The chamber and cover slip are cleaned first with distilled water, then with absolute ethanol, and wiped dry.

Hemacytometer counts do not distinguish between living and dead cells. A number of stains are useful to make this distinction. Trypan Blue, among others (erythrosin B, nigrosin), is excluded by the membrane of the viable cells, whereas the nuclei of damaged or dead cells take up the stain. Although this distinction has been questioned, it has the virtue of being simple and giving a good approximation. If more than 20% of the cells are stained, the result is probably significant.

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Determination of Cell Numbers

Continued

Materials

1. Clean hemacytometer and glass coverslip
2. Pasteur pipettes
3. Hanks' Balanced Salt Solution (HBSS) (Cat. No. 10-543)
4. Trypan Blue, 0.4% in BSS (Cat. No. 17-942E)
5. Microscope
6. Tubes (12 × 75 mm)
7. Hand counter
8. Cell suspension

Procedure

1. Dilute 0.2 mL of Trypan Blue with 0.8 mL of HBSS.
2. Place glass coverslip over hemacytometer chamber.
3. Transfer 0.5 mL of agitated cell suspension to a 12 × 75 mm tube and add 0.5 mL of diluted Trypan Blue.
4. With Pasteur pipette, fill both chambers of the hemacytometer (without overflow) by capillary action. Cells will settle in the tube and in the pipette by gravity within a few seconds. Work quickly.
5. Using a microscope with a 10X ocular and a 10X objective, count the cells in each of 10 squares (1 mm 2 each). If over 10% of the cells represent clumps, repeat entire sequence. If fewer than 200 or more than 500 cells are present in the 10 squares, repeat with a more suitable dilution factor.
6. Calculate the number of cells per mL, and total number of cells in the original culture as follows:

   Cells/mL = average count per square × 10^4 × dilution factor(i.e., 2, if 0.5 mL of cells plus 0.5 mL of Trypan Blue is used)
   Total cells = cells/mL × total volume of cell preparation from which sample was taken
7. Repeat count to check reproducibility.

Powdered Media Preparation

Preparation of Media for Filtration

Powdered media or salt mixtures are extremely hygroscopic and must be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. The preparation of medium in concentrated form is not recommended as some free-base amino acids have low solubility coefficients and insoluble salt complexes may precipitate in concentrated solution. All powdered products do not contain sodium bicarbonate, with the exception of UltraCHO™ Medium (Cat. No. 15-724).
Supplements can be added prior to filtration or introduced aseptically to sterile medium.

NOTE: The nature of the supplement may affect the storage conditions and shelf life of the medium.

Procedure

1. Select a suitable container as close in size to the final volume as possible. Measure out 90% of the final volume of deionized or distilled water (cell culture grade water is available from Lonza in volumes from 500 mL to 200 L).
2. While gently stirring, add the powdered medium or salt mixture. Continue stirring until dissolved. Do not heat the water.
3. Rinse the original package with a small amount of deionized or distilled water. Add to solution.
4. For each liter of final volume of medium being prepared, add to the solution the required amount of sodium bicarbonate and/or L-Glutamine (consult tables below). Continue stirring until completely dissolved.
5. While stirring, adjust the pH to 0.2–0.3 pH units below the desired pH. Use 1 N HCl or 1 N NaOH. The pH will normally rise 0.1–0.3 units during filtration.
6. Add additional deionized or distilled water to bring the medium to final volume.
7. Sterilize immediately by filtration using a 0.22-micron or smaller filter. To reduce the loss of CO_2, positive pressure (3–15 psi), with an inert gas (i.e., nitrogen) should be used for filtering. The use of CO_2 is not recommended, as it will alter the pH of the medium.
8. After filtration, aseptically transfer into sterile containers. Store medium at 2°C to 4°C in the dark until ready to use.

Sodium Bicarbonate Addition Table

Product Description	Cat. No.	$NaHCO_3$ Solution mL/l	$NaHCO_3$ Powder g/l
		17-613	15-613
DMEM with L-Glutamine	15-604	49.3	3.700
DMEM without L-Glutamine	15-614	49.3	3.700
MEM Eagle with L-Glutamine	15-611	29.3	2.200
RPMI with L- Glutamine	15-702	26.7	2.000

L-Glutamine Addition Table

Product Description	Cat. No.	L-Glutamine Solution mL/l	L-Glutamine Powder g/L
		17-605	15-605
DMEM without L-Glutamine	15-614	20.0 (4.0 mM)	0.585 (4.0 mM)

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NOTE: Not applicable to Clonetics™ and Poietics™ Primary Human or Animal Cells.

In cell culture there is frequently the need to subculture cells. In doing so, cells can be propagated for the purposes of increasing cell numbers or providing cells in a culture vessel suitable to one's needs. There are a number of ways to remove cells from one culture vessel and pass them to another vessel. Cells may be removed from surfaces on which they are attached by:

- Mechanical means (scraping)
- Chelating agents, ethylenediaminetetraacetic acid (EDTA)
- Enzymes (trypsin, pronase, collagenase)

Enzymes and chelating agents are often used in combination. Trypsin is an aqueous crude extract prepared from porcine pancreas. It is the most common means used for removal of cells from surfaces and from intact tissue. Trypsin is, to some extent, a misnomer because in addition to trypsin, the preparation contains other proteases, lipases, and carbohydrases. The multitude of digestive enzymes produced by the pancreas would be expected to be found in trypsin preparations. Pure crystalline trypsin can be used, but it is more expensive than crude trypsin and often does not work as well, especially when preparing cells from intact tissue.

The optimum conditions for trypsin activity are a pH range of 7.6–7.8 and a temperature of 37°C. The effect of trypsin is to break down the intracellular matrix that binds cells to each other or to a substrate surface.

There are no chemical standards for trypsin activity. We conduct quality assurance tests on trypsin to determine its capacity to detach cells from a substrate surface in a standard time period without damage. This is in addition to the usual tests for sterility.

Trypsin is typically used at concentrations between 0.05% and 0.25%, although some applications may require concentrations outside this range. Versene® (EDTA) enhances trypsin action, and therefore lowers the required trypsin concentration for effective performance. Concentrated trypsin (2.5%, Cat. No. 17-160) should be diluted in calcium- and magnesium-free balanced salt solution (BSS) (Hanks' BSS, Cat. No. 10-543; or Dulbecco's Phosphate Buffered Saline, Cat. No. 17-512). Dilution in water is not recommended since the solution will be hypotonic and produce cell damage. Dilution in saline alone is also damaging to cells.

Trypsinization Procedure

Cell cultures are normally subcultured ("split") when the cultures are at or near confluency. As a general rule, the longer the time frame between when confluency is first achieved and subculturing, the longer it will take for the trypsin to act.

1. Decant medium from the culture vessel. Serum inhibits trypsin activity, so complete removal of serum-containing medium is necessary.
2. Rinse the cell sheet with BSS without calcium and magnesium before addition of Trypsin/Versene® (Cat. No. 17-161). The monolayer should be thoroughly covered with BSS. This rinse is instantaneous but the BSS can remain on the cell sheet for up to 4 hours, if desired.
3. Pour off rinse medium. Trypsin/Versene® is to be added to each vessel as follows:

75 cm^2 flask	2.5 mL to 5.0 mL
150 cm^2 flask	5.0 mL to 10.0 mL
850 cm^2 roller bottle	10.0 mL to 20.0 mL

4. Cover the monolayer thoroughly with Trypsin/Versene®. Since different lots of Trypsin/Versene® may vary in strength, it is acceptable to monitor the trypsinization process at room temperature for the first 30 seconds. This will ensure that the trypsinization process is not occurring too rapidly.
5. The culture vessel should then be moderately hit against the palm of the hand to see if the cells are being dislodged. Hold the vessel up to a light in a vertical position and look for signs of the cell sheet sloughing off of the surface. If the entire monolayer is dislodged, proceed to step #6. If not, incubate at 37°C and observe the vessel every minute for dissociation. The culture vessel should again be hit against the palm of the hand to ensure all cells have been dislodged. Remove culture vessel from the incubator.

Subculturing Procedures for Mammalian Cells

Continued

6. Immediately transfer dissociated cells to a vessel containing medium supplemented with 10% serum. All of the cells should be removed. Aspirate the medium plus cells with a pipette onto the surface to remove all remaining cells. It is essential that this aspiration be done as completely as possible with a small bore pipette so as to obtain individual, dispersed cells. If the cells are not separated, the new culture will contain numerous microcolonies. Cells added to the vessel should be stirred with a magnetic stir bar at a speed that avoids vortexing (approximately 100–200 rpm), or agitated frequently. It is important at this point to add medium containing serum at least 10 times the volume of Trypsin/Versene® used. This will ensure that the digestive agent is inhibited.
7. Add sufficient fresh medium to the aspirated suspension so that the total volume will cover the surface of two culture vessels, each having the same surface area as the original culture vessel (or use a single culture vessel having twice the floor area of the original vessel). This is a 1:2 split. Other split ratios can be used for vigorously growing cell populations.
8. Incubate the culture vessel (or vessels) at 37°C.
9. When making 1:2 splits, subculturing of human diploid cell cultures should be done on a rigid 3 or 4 day schedule, at which time confluent sheets should occur. Surplus cells can be frozen and stored in liquid nitrogen.
10. Populations that can be cultivated indefinitely can be subcultured serially each time confluency is reached. If the culture is a diploid population with a finite doubling capacity, increase the population doubling level (PDL) number by one at each 1:2 subculturing (split).
11. By making repeated 1:2 splits (twice a week) it can be seen that the number of culture vessels can be built up geometrically (1, 2, 4, 8, 16, 32, 64, etc.) in a short period of time for the production of large quantities of cells for various purposes.
12. Although the line will be eventually lost as a continuously passaged line, it will not be lost for use since frozen ampoules can be obtained at almost every passage and thus the line can be restored to continuous passage again, up to a cumulative total of about 50 population doublings. By repeating this procedure, the number of cells that can be obtained is almost unlimited for all practical purposes.
13. A human embryonic diploid line has an in vitro life span of about fifty 1:2 subcultivations, or population doublings, at which time the cells will cease to divide and eventually die.
14. Using split ratios higher than 1:2 results in the advantage of minimizing the number of manipulations necessary to obtain a specific cell density or number of culture vessels. Since human embryonic diploid cell lines pass through a finite number of population doublings in vitro, it is necessary to keep a record of the number of population doublings that have elapsed. With a 1:2 split ratio this is achieved by simply adding "1" to each split since this ratio yields one population doubling. Larger split ratios can be used. For example, a split ratio of 1:4 would yield 2 doublings per 1:4 split; a 1:8 split ratio would yield 3 doublings per 1:8 split. In order to have knowledge of the approach of cessation, it is essential to keep records of the number of elapsed population doublings.
15. Since human diploid cells multiply by fission, the increase in population may be expressed per cell as follows:

1	2	4	8	16	…Number of cells
0	1	2	3	4	…Population Doubling Level

References

1. **Hayflick, L.** and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Research 25:585.
2. **Hayflick, L.** (1970) Aging under glass. Exp. Geront. 5:291.
3. **Hayflick, L.** (1965) The limited *in vitro* lifetime of human diploid cell strains. Exp. Cell Res. 37:614.
4. **Hayflick, L.** (1968) Human cells and aging. Scientific American 218:32.
5. **Hayflick, L.** (1973) Subculturing human diploid fibroblast cultures. Methods and Applications of Tissue Culture Eds. Patterson, M.K. and Kruse, P.F., Academic Press, N.Y.
6. **Freshney, R.I.** (1983) Culture of Animal Cells: A Manual of Basic Technique. Alan R. Liss, Inc., New York.

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Frequently Asked Questions – Nucleic Acid

Electrophoresis and Agarose

Q. What buffer conditions give me best resolution for agarose electrophoresis?

A. For small DNA fragments (<1,000 bp) when recovery is not necessary, we recommend the use of 1X TBE Buffer. Gels made with TBE Buffer give sharper bands than gels made with TAE Buffer. TBE results in better resolution for closely spaced DNA bands.

For large DNA fragments (>15,000 bp), 1X TAE Buffer enhances separation of large DNA. Since TAE has a lower buffering capacity, it may be necessary either to recirculate the buffer, or periodically mix the buffer between the anodal and cathodal chambers when electrophoresing for an extended period of time. The time to buffer depletion can vary with the volts/hour and the size of chamber used.

Buffer depth over the gel should be 3 to 5 mm deep. Less buffer, and you risk the chance of the gel drying out. Excessive buffer will decrease the resistance of the circuit between the anode and cathode, which results in a decreased voltage gradient through the gel. This causes inefficient DNA mobility, excessive heating, and band distortion.

Q. How should I cast my gels to get the best resolution?

A. We usually cast gels 3 mm to 4 mm thick. The gel volume needed can easily be estimated by measuring the surface area of the casting chamber, then multiplying by the gel thickness. Thinner gels can be cast on GelBond® Support Film, and/or cast in a vertical apparatus.

The thickness of the comb in the direction of the electrical field can also profoundly affect the resolution. A thin comb (1 mm) will result in sharper DNA bands. With too thick a comb, the separated DNA bands will be quite broad.

Q. My DNA bands are sometimes wavy, but usually only in one or two lanes. What causes this?

A. Dried agarose on the comb teeth is a frequent cause of this problem. Prior to casting your gel, check the comb teeth for residual dried agarose. If not removed, this will attach to the newly cast agarose and fracture the well upon comb removal. This is usually not observable until the gel is on the transilluminator. Additionally, care must be taken during comb removal, particularly with low melting temperature agaroses. Well integrity may be maintained in these agaroses by pre-chilling the gel to 4°C for 30 minutes and/or by flooding the gel with cold buffer prior to removing the comb.

Q. How much DNA should I load per well?

A. The amount to load per well is variable. What is most important is how much DNA there is in the bands you wish to resolve. The least amount of DNA that can be consistently detected with ethidium bromide is about 10 ng. The most DNA you can have in a band and still get a sharp, clean band on an ethidium bromide stained gel is about 100 ng. These amounts will be less on gels stained with more sensitive stains such as GelStar® Stain. On a GelStar® Stained Gel it is possible to detect as little as 20 pg dsDNA.

The optimal amount of DNA to load in the well is calculated by the fraction of the total DNA which is in the band of interest. If you are unsure of how much DNA is present, load varying amounts in several lanes if possible.

The optimal amount of DNA to load in the well may be calculated by the fraction of the total DNA which is in the band of interest, represented by the following:

$$\frac{\text{Fragment of interest (kbp)}}{\text{Size of DNA sample (kbp)}} \times 100 = \text{\% DNA in band of interest}$$

NOTE: The most DNA compatible with a clean sharp band is approximately 100 ng.

For example:

The size of your DNA sample is 48.5 kbp and when run on the gel 8 fragments are separated. Your fragment of interest is 2.3 kbp.

Calculation:

$$\frac{2.3 \text{ kbp}}{48.5 \text{ kbp}} \times 100 = 4.7\% \text{ DNA in fragment of interest}$$

If you load 1 µg of DNA, then 4.7% of the 1 µg of loaded sample will appear in your fragment of interest (47 ng). To further increase the sharpness of the bands, use a Ficoll® Based Loading Buffer, such as Lonza DNA Loading Buffer (Cat. No. 50655) instead of sucrose-based or glycerol-based loading buffers. The use of lower molecular weight glycerol will allow DNA to stream up the sides of the well before electrophoresis which results in U-shaped bands.

Frequently Asked Questions

Continued

Loading buffer that is too high in ionic strength can cause the bands to be fuzzy. In the ideal situation, the DNA sample should be suspended in the same solution as the running buffer. If this is not possible, use a sample buffer with a lower ionic strength than the running buffer.

Q. At what voltage should I run an agarose gel?

A. We recommend running agarose gels at 4–10 volts/cm (cm is determined by measuring the interelectrode distance, not the gel length) under normal horizontal electrophoretic conditions. If the voltage is too high, band streaking, especially for DNA >15 kb, may result. When the voltage is too low, the mobility of small (<1,000 bp) DNA is reduced and band broadening will occur due to diffusion.

MetaPhor™ Agarose Gels separate DNA optimally at 4.5–5 volts/cm in standard horizontal electrophoresis systems. Higher voltages result in a decrease in the resolution of DNA separation, mainly due to gel overheating.

Another special case is the separation of large (>15 kb) DNA fragments using conventional horizontal electrophoresis. The best separations in this instance are obtained at a voltage gradient of <5 volts/cm.

Q. What is the difference between NuSieve™ 3:1 and NuSieve™ GTG™ Agaroses?

A. NuSieve™ 3:1 Agarose is a standard melting temperature agarose. The resolution range forNuSieve™ 3:1 Agarose is 50 bp–1000 bp. NuSieve™ 3:1 Agarose is designed for analytical electrophoresis; its high gel strength also makes it ideal for use in various blotting techniques. NuSieve™ GTG™ Agarose is a low melting temperature agarose (≤65°C at 4%). The resolution range for this agarose is 50 bp–1000 bp. NuSieve™ GTG™ Agarose is recommended for in-gel applications such as cloning or ligation and transformation.

Q. What is a GTG™ Agarose?

A. GTG™ stands for Genetic Technology Grade™. GTG™ Grade Agarose is recommended for preparative DNA electrophoresis, or when further enzymatic manipulation of DNA is required. These agaroses are extensively tested to ensure maximum compatibility with standard molecular biology techniques.

Precast Agarose Gels

Q. Do I need to purchase a special chamber to use Reliant™ and Latitude™ Precast Gels?

A. Reliant™ and Latitude™ Precast Agarose Gels are designed to run in standard horizontal electrophoresis chambers. As long as there is room on the chamber platform for the gel, the chamber should be suitable. Measure the chamber platform and check against precast gel size to be sure. For example, the OWL® Centipede™ Chamber is ideal with the 14 cm × 24 cm Latitude™ HT Precast Gels; the OWL® B1 EasyCast™ Chamber is good with Reliant™ Precast Agarose Gels; and the Latitude™ Chamber is perfect with Latitude™ Midigels. Results with different chambers will vary depending on differences in chamber size and construction.

Q. Are the materials in the FlashGel™ Cassette hazardous?

A. The stain in the FlashGel™ Cassette is present at such low levels that it is not considered hazardous according to OSHA and EU hazard criteria. A copy of the MSDS is available online. The stain in the cassette is a potential mutagen. Wear gloves, safety glasses and a lab coat when handling. Use the same precautions when handling and disposing of the cassettes as you would ethidium bromide stained gels.

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PAGEr™ Precast Gels

Q. Which PAGEr™ Precast Gels will fit my gel chamber?

A. PAGEr™ Precast Gels are available in 9 cm × 10 cm and 10 cm × 10 cm sizes and fit most standard mini-vertical systems. Some chambers may require modifications for optimal fit with PAGEr™ Precast Gels.

Standard Vertical Systems	PAGEr™ Gels
PAGEr™ Minigel Chamber	9 cm × 10 cm or 10 cm × 10 cm gels*
Bio-Rad® Mini-PROTEAN® II, Mini-PROTEAN® 3 , Mini-PROTEAN® Tetra, Mini-PROTEAN® Dodeca™ and Ready Gel® Cell Systems. Reverse the inner core gasket so the flat side faces outward.	9 cm × 10 cm gels
Novex® XCell SureLock® Mini-Cell or XCell II Request the spacer for the XCell SureLock® Mini-Cell Chamber from Scientific Support, (Cat. No. 59900).	9 cm × 10 cm or 10 cm × 10 cm gels*
FisherBiotech® Vertical Minigel FBVE121, Owl Separations Systems Wolverine™ P82 Chamber comes with 2 sets of wedges. Use the thinner wedges for the PAGEr™ Gold Gels.	10 cm × 10 cm gels
FisherBiotech® Vertical Minigel FB-VE101, Owl Separations Systems Penguin™ Model P8DS Request adaptor for these chambers from Scientific Support, (Cat. No. 59902).	10 cm × 10 cm gels
Hoefer® Mighty Small™ (SE250, SE260) If using SE250 replace the buffer chamber with a 'Deep lower buffer chamber for the SE260', order number 80-6148-78, from GE Healthcare.	9 cm × 10 cm or 10 cm × 10 cm gels*
Daiichi 2, ISS chambers	10 cm × 10 cm gels
Hoefer® Mighty Small™ (SE260)	9 cm × 10 cm or 10 cm × 10 cm gels
EC 120 Mini Vertical Gel System	9 cm × 10 cm or 10 cm × 10 cm gels
Biometra® Mini V Chamber	9 cm × 10 cm gels
CBS Scientific MGV System, (10 cm × 8 cm units)	9 cm × 10 cm gels
Sigma-Aldrich Mini Techware (11.3 cm × 10 cm units)	10 cm × 10 cm gels
Zaxis System 2000	10 cm × 10 cm gels
Hoefer® Mini VE	10 cm × 10 cm gels

*Recommended for best fit

Q: Do Lonza PAGEr™ Precast Gels contain a stacking gel? What is the purpose of the stacking gel?

A. PAGEr™ Gold Precast Gels contain a 4% stacking gel, pH 8.6. The purpose of this stacking gel is to allow the proteins to accumulate and condense (i.e., stack) at the stacking/resolving gel boundary. This stacking effect results in superior resolution.

Q. I would like to run a native or nondenaturing gel. What can I use?

A. PAGEr™ Precast Gels do not contain SDS or any other denaturing agents (e.g., DTT and b-ME). Additionally, you would use a Tris-Glycine Running Buffer that does not contain SDS.

Frequently Asked Questions

Continued

Protein Electrophoresis

Q. How do I make the transfer, running, and sample buffers?

A. Tris-Glycine Gels (Tris-HCl Buffer System)

Towbin transfer buffer (1X)	Running buffer (1X)	Sample buffer (1X)
0.025 M Tris base	25 mM Tris Base	62.5 mM Tris-HCl, pH 6.8
0.192 M Glycine	192 mM Glycine	2% SDS*
0.05–0.1% SDS*	0.1% SDS*	10% Glycerol
20% Methanol		0.01% Bromophenol Blue
		2.5% bME (2-mercaptoethanol)*

*Omit for native proteins.
For best results use Lonza AccuGENE™ Electrophoresis Buffers or use ProSieve™ EX Running Buffer for fast denaturing protein separation. ProSieve™ EX Running Buffer is required with PAGEr™ EX Gels.

Q. What is the difference between gradient vs. homogeneous (single concentration) gels? Which one should I use?

A. Gradient gels are suitable for a wide range of size resolutions. A homogeneous or single concentration gel is appropriate where the proteins of interest are known to be within a narrow size range.

Q. How much protein should I load on the gel?

A. Protein load levels will vary depending upon sample purity and staining method used. For highly purified proteins, 0.5 µg to 5 µg protein per lane on a minigel is generally sufficient. Complete mixtures such as cell lysates may require as much as 50 µg protein per lane.

Protein Stain Detection Limits

Protein stain	Lower detection limit (protein/band)
Coomassie® Blue Stain	30 ng
Silver Stain	2 ng
SYPRO® Red Protein Gel Stain	4 ng–8 ng
SYPRO® Ruby Protein Gel Stain	2 ng–8 ng
SYPRO® Tangerine Protein Gel Stain	4 ng–8 ng
ProSieve™ EX Safe Stain	8 ng–15 ng

NOTE: Limits are based on optimal detection methods for each stain.

Q. What is the best membrane to use for Western blotting?

A. Use this table to find a suitable membrane.

Nitrocellulose	PVDF	Nylon
Hydrophobic binding	Hydrophobic binding	Hydrophobic and electrostatic binding
General purpose membrane	SDS tolerant	Stable if baked
Low background	High background	High background
Low strength	High strength	High strength
Becomes brittle if baked	Suitable for protein sequencing	Least suitable for Western transfer

Q. What are the benefits of using agarose for protein gel electrophoresis?

A. Protein electrophoresis in agarose gels is an alternative approach to using polyacrylamide gels and provides several benefits:

- Separate high molecular weight proteins (>600 kDa)
- Easy to prepare and handle
- Efficient recovery of proteins
- Excised proteins can be used to immune animals directly for antibody production
- Non-toxic
- Run gels using either a vertical or horizontal apparatus

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Agarose Types

The appropriate choice of agarose depends on the size of the DNA to be analyzed and any subsequent manipulations required.Gelling/meltingtemperatures,electroendoosmosis and gel strength are all important factors in choosing the right agarose for your application. Refer to page 456 for analytical specifications of Lonza Agarose.

Genetic Technology Grade™ (GTG™) Agaroses

Our Genetic Technology Grade™ (GTG™) Agarose Products are specially prepared and certified for demanding molecular biology applications for nucleic acids, including PCR amplified products. Our GTG™ Agarose quality tests go beyond standard assays, such as DNase and RNase testing, to include enzymatic performance measurements. Our additional testing provides a more realistic index of overall product quality and reliability. You no longer need to screen agarose lots to find those that yield biologically active DNA.

The following agaroses are GTG™ Certified:

- SeaKem® GTG™ Agarose
- SeaPlaque™ GTG™ Agarose (low melting temperature agarose)
- NuSieve™ GTG™ Agarose (low melting temperature agarose)
- SeaKem® Gold Agarose

We perform the following tests on GTG™ Certified Agaroses:

- DNA binding
- DNase and RNase activity
- DNA resolution
- Gel background-gel exhibits low background fluorescence after ethidium bromide staining
- In-gel cloning (low melt agarose)
- In-gel restriction digestion (low melt agarose)
- Restriction-ligation assay (SeaKem® GTG™)

Molecular Biology Grade Agaroses

Molecular biology grade agaroses are suitable for general analytical separation of DNA.

The following agaroses are considered molecular biology grade agaroses:

- MetaPhor™ Agarose
- SeaKem® LE Agarose
- NuSieve™ 3:1 Agarose
- SeaPlaque™ Agarose (low melting temperature agarose)

We screen our molecular biology grade agaroses for the following parameters:

- DNA binding
- DNase and RNase activity
- Gel background staining

FDA Listing

Our agarose types are listed as Class 1 Medical Devices under registration number 1219614.

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Preparation of Agarose Gels

Suggested Agarose Concentrations and Dye Migration Information

Table 1: Suggested Agaroses for Particular Applications

Size Range (base pairs)	Agarose Type	Application
20–800	MetaPhor™ Agarose	High resolution analysis; 2% size differences
50–1,000	NuSieve™ 3:1 Agarose	Analysis and blotting; 4%–6% size differences resolved
	NuSieve™ GTG™ Agarose	Analysis and blotting; In-gel; 6% size differences resolved
1,000–10,000	SeaKem® GTG™ Agarose	Analysis and blotting; recovery required
	SeaPlaque™ GTG™ Agarose	In-gel
10,000	SeaKem® Gold Agarose	Analysis

Table 2: Properties of TAE and TBE Buffer Systems

Buffer	Suggested Uses and Comments
TAE buffer	Use when DNA is to be recovered Use for electrophoresis of large (>12 kb) DNA Low ionic strength Low buffering capacity – recirculation may be necessary for extended electrophoretic times
TBE buffer	Use for electrophoresis of small (<1 kb) DNA Decreased DNA mobility High ionic strength High buffering capacity – no recirculation required for extended run times

Agarose Analytical Specifications

	Agarose	Melting Temperature	Gel Strength g/cm^2	Gelling Temperature	EEO (-mr)	Moisture	Sulfate
DNA <1 kb	NuSieve™ 3:1	≤90°C at 4%	≥1,400 at 4%	32.5°C–38°C at 4%	≤0.13	≤10%	≤0.15%
	MetaPhor™	≤75°C at 3%	≥300 at 3%	≤35°C at 3%	≤0.05	≤10%	n/a
	NuSieve™ GTG™	≤65°C at 4%	≥500 at 4%	≤35°C at 4%	≤0.15	≤10%	≤0.15%
DNA >1 kb	SeaKem® LE	NA	≥1,200 at 1%	36°C ± 1.5°C at 1.5%	0.09–0.13	≤10%	≤0.15%
	SeaKem® GTG™	NA	≥1,200 at 1%	36°C ± 1.5°C at 1.5%	0.09–0.13	≤10%	≤0.15%
	SeaPlaque™	≤65°C at 1.5%	≥200 at 1%	26°C–30°C at 1.5%	≤0.10	≤10%	≤0.10%
	SeaPlaque™ GTG™	≤65°C at 1.5%	≥200 at 1%	26°C–30°C at 1.5%	≤0.10	≤10%	≤0.10%
PFGE	SeaKem® Gold	NA	≥1,800 at 1%	34.5°C –37.5°C at 1.5%	≤0.05	≤10%	≤0.10%
			≥3,500 at 1.5%				
	InCert™	≤70°C at 1.5%	≥350 at 1%	26°C–30°C at 1.5%	≤0.10	≤10%	≤0.15%
Identity testing	I.D.NA™	NA	≥1,300 at 1%	36°C ± 1.5°C at 1.5%	≤0.10	≤10%	≤0.15%
Protein electrophoresis	SeaKem® ME	NA	≥1,000 at 1%	36°C ± 1.5°C at 1.5%	0.16–0.19	≤10%	≤0.20%
	SeaKem® HE	NA	≥650 at 1%	36°C ± 1.5°C at 1.5%	0.23–0.26	≤10%	≤0.20%
	SeaKem® HEEO	NA	≥650 at 1%	36°C ± 1.5°C at 1.5%	≥0.30	≤10%	≤0.25%
	SeaKem® HGT	NA	≥800 at 1%	42°C ± 1.5°C at 1.5%	≤0.10	≤10%	≤0.30%
Isoelectric focusing	IsoGel™	NA	≥500 at 1.5%	35°C–45°C	Not Detectable	≤10%	≤0.20%
Cell culture	SeaPrep™	≤50°C at 1%	≥75 at 2%	8°C–17°C at 0.8%	≤0.05	≤10%	≤0.10%

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Preparation of Agarose Gels

Continued

Table 3: Suggested Agarose Concentrations for DNA Sizes

Size range (base pairs)	Final Agarose Concentration % (w/v)	
	1X TAE buffer	1X TBE buffer
SeaKem® LE and SeaKem® GTG™ Agarose		
1,000–23,000	0.60	0.50
800–10,000	0.80	0.70
400–8,000	1.00	0.85
300–7,000	1.20	1.00
200–4,000	1.50	1.25
100–3,000	2.00	1.75
NuSieve™ 3:1 Agarose		
500–1,000	3.0	2.0
100–500	4.0	3.0
10–100	6.0	5.0
MetaPhor™ Agarose		
150–800	2.0	1.8
100–600	3.0	2.0
50–250	4.0	3.0
20–130	5.0	4.0
<80	–	5.0
SeaPlaque™ and SeaPlaque™ GTG™ Agarose		
500–25,000	0.75	0.70
300–20,000	1.00	0.85
200–12,000	1.25	1.00
150–6,000	1.50	1.25
100–3,000	1.75	1.50
50–2,000	2.00	1.75
NuSieve™ GTG™ Agarose		
500–1,000	2.5	2.0
150–700	3.0	2.5
100–450	3.5	3.0
70–300	4.0	3.5
10–100	4.5	4.0
8–50	5.0	4.5
SeaKem® Gold Agarose†		
5,000–50,000	0.3	–
1,000–20,000	0.5	–
800–10,000	0.8	–
400–8,000	1.0	–

† TBE buffer is not recommended for separation of DNA >12,000 bp.

Table 4: Migration of Double-stranded DNA in Relation to Bromophenol Blue (BPB) and Xylene Cyanol (XC) in Agarose Gels

	1X TAE Buffer		% Agarose	1X TBE Buffer	
	XC	BPB		XC	BPB
SeaKem® LE and SeaKem® GTG™ Agarose					
	24,800	2,900	0.30	19,400	2,850
	16,000	1,650	0.50	12,000	1,350
	10,200	1,000	0.75	9,200	720
	6,100	500	1.00	4,100	400
	3,560	370	1.25	2,500	260
	2,800	300	1.50	1,800	200
	1,800	200	1.75	1,100	110
	1,300	150	2.00	850	70
NuSieve™ 3:1 Agarose					
	950	130	2.50	700	70
	650	80	3.00	500	40
	350	40	4.00	250	20
	200	30	5.00	140	8
	120	20	6.00	90	4
MetaPhor™ Agarose					
	480	70	2.00	310	40
	200	40	3.00	140	35
	120	35	4.00	85	30
	85	30	5.00	60	15
SeaPlaque™ and SeaPlaque™ GTG™ Agarose					
	11,700	1,020	0.50	6,100	400
	4,000	500	0.75	2,850	280
	2,300	350	1.00	1,700	180
	1,500	200	1.25	1,000	100
	1,000	150	1.50	700	70
	700	100	1.75	500	50
	550	60	2.00	400	30
	320	30	2.50	250	10
NuSieve™ GTG™ Agarose					
	750	175	2.50	460	75
	400	120	3.00	210	35
	115	<20	4.00	150	<20
	100	<20	5.00	80	<20
	85	<20	6.00	50	<20
SeaKem® Gold Agarose					
	24,800	3,550	0.30	19,000	2,550
	12,200	2,050	0.50	9,200	1,500
	9,200	1,050	0.75	7,100	800
	6,100	760	1.00	4,000	500
	4,100	600	1.25	2,550	350
	2,600	400	1.50	1,900	250
	2,000	330	1.75	1,400	180
	1,500	250	2.00	1,000	100

Preparation of Agarose Gels

Dissolving Agarose

Agarose undergoes a series of steps when it is dissolved: dispersion, hydration and melting/dissolution.

Microwave Instructions for Gel Concentrations <2%w/v

1. Choose a beaker that is 2–4 times the volume of the solution.
2. Add room temperature 1X or 0.5X buffer and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred.
4. Remove the stir bar if not Teflon®-coated.
5. Weigh the beaker and solution before heating.
6. Cover the beaker with plastic wrap.
7. Pierce a small hole in the plastic wrap for ventilation.
8. Heat the beaker in the microwave oven on **high** power until bubbles appear.

⚠ CAUTION: Any microwaved solution may become superheated and foam over when agitated.

9. Remove the beaker from the microwave oven.
10. **Gently** swirl the beaker to resuspend any settled powder and gel pieces.
11. Reheat the beaker on **high** power until the solution comes to a boil.
12. Hold at boiling point for 1 minute or until all of the particles are dissolved.
13. Remove the beaker from the microwave oven.

⚠ CAUTION: Use oven mitts when removing beaker from microwave, as container will be hot and may cause burns.

14. **Gently** swirl the beaker to mix the agarose solution thoroughly.
15. After dissolution, add sufficient hot distilled water to obtain the initial weight.
16. Mix thoroughly.
17. Cool the solution to 60°C prior to casting.

Materials

- Microwave oven or hot plate
- Beaker that is 2–4 times the volume of the solution
- Teflon®-coated magnetic stir bar
- Magnetic stir plate
- Plastic wrap
- Oven mitts or other heat protection for hands

Reagents

- Distilled water
- Agarose powder

⚠ CAUTION: Always wear eye protection, and guard yourself and others against scalding solutions.

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Preparation of Agarose Gels

Continued

Microwave Instructions for Gel Concentrations ≥2% w/v

1. Choose a beaker that is 2–4 times the volume of the solution.
2. Add room temperature or chilled buffer (for MetaPhor™ and NuSieve™ GTG™ Agarose) and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred to prevent the formation of clumps.
4. Remove the stir bar if not Teflon®-coated.
5. Soak the agarose in the buffer for 15 minutes before heating. This reduces the tendency of the agarose solution to foam during heating.
6. Weigh the beaker and solution before heating.
7. Cover the beaker with plastic wrap.
8. Pierce a small hole in the plastic wrap for ventilation. For agarose concentrations >4%, the following additional steps will further help prevent the agarose solution from foaming during melting/dissolution:
   - 8.1 Heat the beaker in the microwave oven on **medium** power for 1 minute.
   - 8.2 Remove the solution from the mircrowave.
   - 8.3 Allow the solution to sit on the bench for 15 minutes.
9. Heat the beaker in the microwave oven on **medium** power for 2 minutes.

⚠ CAUTION: Any microwaved solution may become superheated and foam over when agitated.

10. Remove the beaker from the microwave oven.

⚠ CAUTION: Use oven mitts when removing beaker from microwave, as container will be hot and may cause burns.

11. **Gently** swirl to resuspend any settled powder and gel pieces.
12. Reheat the beaker on **high** power for 1–2 minutes or until the solution comes to a boil.
13. Hold at the boiling point for 1 minute or until all of the particles are dissolved.
14. Remove the beaker from the microwave oven.
15. **Gently** swirl to mix the agarose solution thoroughly.
16. After dissolution, add sufficient hot distilled water to obtain the initial weight.
17. Mix thoroughly.
18. Cool the solution to 60°C prior to gel casting.

Hot Plate Instructions for Preparing Agarose

1. Choose a beaker that is 2–4 times the volume of the solution.
2. Add room temperature or chilled buffer (for MetaPhor™ or NuSieve™ GTG™ Agarose) and a stir bar to the beaker.
3. Sprinkle in the premeasured agarose powder while the solution is rapidly stirred to prevent the formation of clumps.
4. Weigh the beaker and solution before heating.
5. Cover the beaker with plastic wrap.
6. Pierce a small hole in the plastic wrap for ventilation.
7. Bring the solution to a boil while stirring.
8. Maintain gentle boiling until the agarose is dissolved (approximately 5–10 minutes).
9. Add sufficient hot distilled water to obtain the initial weight.
10. Mix thoroughly.
11. Cool the solution to 60°C prior to casting.

⚠ CAUTION: Always wear eye protection, and guard yourself and others against scalding solutions.

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Preparation of Agarose Gels

Continued

Horizontal Gel Casting Instructions

1. Allow the agarose solution to cool to 60°C.
2. While the agarose solution is cooling:
   - 2.1 Assemble the gel casting tray.
   - 2.2 Level the casting tray prior to pouring the agarose solution.
   - 2.3 Check the teeth of the comb(s) for residual dried agarose. Dried agarose can be removed by scrubbing the comb teeth with a lint-free tissue soaked in hot distilled water.
   - 2.4 Allow a small space (approximately 0.5 mm–1 mm) between the bottom of the comb teeth and the casting tray.
3. Pour the agarose solution into the gel tray.
4. Replace the comb(s).
5. Allow the agarose to gel at room temperature for 30 minutes.
6. Low melting temperature agaroses and MetaPhor™ Agarose require an additional 30 minutes of gelling at 4°C to obtain the best gel handling. The additional cooling step is essential for obtaining fine resolution in MetaPhor™ Agarose.
7. Once the gel is set, flood with running buffer.
8. Slowly remove the comb.
9. Place the gel casting tray into the electrophoresis chamber.
10. Fill the chamber with running buffer until the buffer reaches 3 mm–5 mm over the surface of the gel.
11. Gently flush the wells out with electrophoresis buffer using a Pasteur pipette to remove loose gel fragments prior to loading the samples.
12. Load DNA and electrophorese.

The thickness of the comb in the direction of the electric field can affect the resolution. A thin comb (1 mm) will result in sharper DNA bands. With a thicker comb, more volume can be added to the well but the separated DNA bands may be broader.

Materials

- Horizontal electrophoresis apparatus
- Combs
- Pasteur pipette

Reagents

- Agarose solution
- Electrophoresis buffer

Voltage Table

The table below provides a quick reference for optimal voltage for DNA electrophoresis.

Recommended Voltages and Buffers Related to DNA Size and Application

Size	Voltage	– – Buffer– –	
		Recovery	Analytical
≤1 kb	5 V/cm	TAE	TBE
1 kb to 12 kb	4–10 V/cm	TAE	TAE/TBE
>12 kb	1–2 V/cm	TAE	TAE

Optimal Electrophoretic Time

The gel should be run until the band of interest has migrated 40%–60% down the length of the gel (see the Dye Mobility Table, see page 457). Band broadening resulting from dispersion and diffusion results in a decrease in resolution in the lower third of the gel. Resolution may also be decreased in smaller gels, since longer electrophoretic runs result in greater separation between two fragments.

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Loading Buffers

Gel loading buffers serve three purposes in DNA electrophoresis:

- Increase the density of the sample: This ensures that the DNA will drop evenly into the well
- Add color to the sample: Simplifies loading
- Add mobility dyes: The dyes migrate in an electric field towards the anode at predictable rates. This enables one to monitor the electrophoretic process

Types of Loading Buffers

At least five loading buffers are commonly used for agarose gel electrophoresis. They are prepared as six-fold concentrated solutions. If needed, 10X solutions of each buffer can also be prepared. Alkaline loading buffer is used when performing alkaline gel electrophoresis.

Loading Buffer	6X recipe	Storage Temperature
Sucrose-based	40% (w/v) Sucrose 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	4°C
Glycerol-based	30% Glycerol in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	4°C
Ficoll®-based	15% Ficoll® (Type 400) Polymer in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	room temperature
Alkaline	300 mN NaOH 6 mM EDTA 18% Ficoll® (Type 400) Polymer in distilled water 0.15% Bromocresol Green 0.25% Xylene cyanol FF	4°C

Ficoll®-Based Loading Buffers

To increase the sharpness of DNA bands, use Ficoll® (Type 400) Polymer as a sinking agent instead of glycerol. The use of the lower molecular weight glycerol in the loading buffer allows DNA to stream up the sides of the well before electrophoresis has begun and can result in a U-shaped band. In TBE gels, glycerol also interacts with borate, which can alter the local pH.

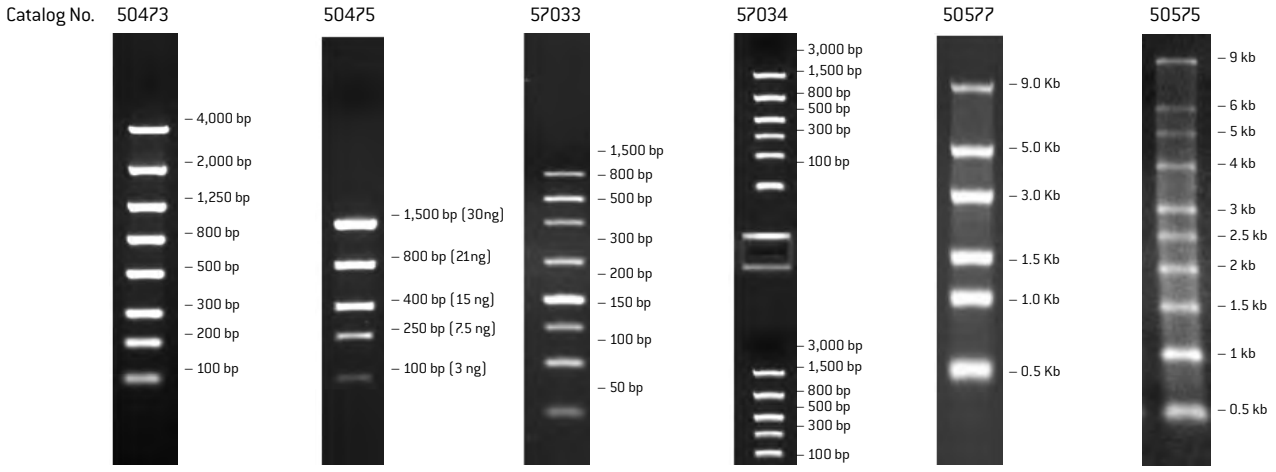
Sample Preparation

Loading buffer that is too high in ionic strength causes bands to be fuzzy and migrate through the gel at unpredictable rates. Ideally, the DNA sample should be resuspended in the same solution as the running buffer. If this is not possible, use a sample buffer with a lower ionic strength than the running buffer.

Detection and Sizing of DNA in Agarose Gels

FlashGel™ DNA and RNA Markers

Size Range	0.5 Kb – 9.0 kb	0.5 Kb – 9.0 kb	100 bp–3 kb	100 bp–4 kb	100 bp–1.5 kb	50 bp–1.5 kb
Ladder/marker	RNA marker	RNA marker	DNA marker	DNA marker	Quant ladder	DNA marker
Cat. No.	50575	50577	57034	50473	50475	57033
Number of bands	10	6	6	8	5	8
	9.0 kb	9.0 kb	3,000 bp	4,000 bp	1,500 bp	1,500 bp
	6.0 kb	5.0 kb	1,500 bp	2,000 bp	800 bp	800 bp
	5.0 kb	3.0 kb	800 bp	1,250 bp	400 bp	500 bp
	4.0 kb	1.5 kb	500 bp	800 bp	250 bp	300 bp
			300 bp			
	3.0 kb	1.0 kb	100 bp	500 bp	100 bp	200 bp
	2.5 kb	0.5 kb		300 bp		150 bp
	2.0 kb			200 bp		100 bp
	1.5 kb			100 bp		50 bp
	1.0 kb					
	0.5 kb					



FlashGel™ DNA Marker
100 bp–4 kb
1.2%, 12 + 1
(Standard)

FlashGel™ DNA Marker
100 bp–1.5 kb
1.2%, 12 + 1
(Quantitation)

FlashGel™ DNA Marker
50 bp–1.5 kb
2.2%, 12 + 1
(High Concentration)

FlashGel™ DNA Marker
100 bp–3 kb
1.2%, 16 + 1
(Double-tier)

FlashGel™ RNA Marker
0.5 kb–9 kb
1.2%, 12 + 1 & 16 +1
(RNA)

Lonza RNA Marker
0.5 bp–9 kb
1.2%, 12 + 1
(RNA)

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Detection and Sizing of DNA in Agarose Gels

Continued

Guide to Lonza Ladders and Markers – Size Range (Bold numbers indicate brighter bands)

	20 bp	20 bp Ext	100 bp	100 bp ext	Tandem	500 bp	Quant Ladder	Rev Quant Ladder	50 bp – 1000 bp	50 bp – 2500 bp	1 kb – 10 kb
Standard ladders											
Cat. No.	50330	50320	50321	50322	NA	50323	50334	50335	50461	50631	50471
SimplyLoad™ ladders											
Cat. No.	50331	50326	50327	50328	50333	50329	50336	50337	NA	NA	NA
Number of fragments	25	50	100	30	21	16	5	5	9	13	9
Size range	20 bp–500 bp	20 bp–1,000 bp	100 bp–1,000 bp	10 bp–3,000 bp	100 bp–12 kb	500 bp–8 kb	100 bp–1,000 bp	100 bp–1,000 bp	50 bp–1,000 bp	50 bp–2,500 bp	1 kb – 10 kb
	500 bp	1,000 bp	1,000 bp	**3,000 bp**	12 kb	8,000 bp	1,000 bp	1,000 bp	1,000 bp	2.5 kb	10 kb
	480 bp	980 bp	900 bp	2,900 bp	11 kb	7,500 bp	700 bp	700 bp	700 bp	2 kb	7 kb
	460 bp	960 bp	800 bp	2,800 bp	10 kb	7,000 bp	500 bp	500 bp	525 bp	1.5 kb	5 kb
	440 bp	940 bp	700 bp	2,700 bp	9 kb	6,500 bp	200 bp	200 bp	500 bp	1250 bp	4 kb
	420 bp	920 bp	600 bp	2,600 bp	8 kb	6,000 bp	100 bp	100 bp	400 bp	**1 kb**	3 kb
	400 bp	900 bp	500 bp	2,500 bp	7 kb	5,500 bp			300 bp	700 bp	2.5 kb
	380 bp	880 bp	400 bp	2,400 bp	6 kb	**5,000 bp**			200 bp	525 bp	2 kb
	360 bp	860 bp	300 bp	2,300 bp	**5 kb**	4,500 bp			100 bp	500 bp	1.5 kb
	340 bp	840 bp	200 bp	2,200 bp	4 kb	4,000 bp			50 bp	400 bp	1 kb
	320 bp	820 bp	100 bp	2,100 bp	3 kb	3,500 bp				300 bp	
	300 bp	800 bp		2,000 bp	2 kb	3,000 bp				200 bp	
	280 bp	780 bp		1,900 bp	**1 kb**	2,500 bp				100 bp	
	260 bp	760 bp		1,800 bp	900 bp	2,000 bp				50 bp	
	240 bp	740 bp		1,700 bp	800 bp	1,500 bp					
	220 bp	720 bp		1,600 bp	700 bp	1,000 bp					
	200 bp	700 bp		1,500 bp	600 bp	500 bp					
	180 bp	680 bp		1,400 bp	500 bp						
	160 bp	660 bp		1,300 bp	400 bp						
	140 bp	640 bp		1,200 bp	300 bp						
	120 bp	620 bp		1,100 bp	200 bp						
	100 bp	600 bp		**1,000 bp**	100 bp						
	80 bp	580 bp		900 bp							
	60 bp	560 bp		800 bp							
	40 bp	540 bp		700 bp							
	20 bp	520 bp		600 bp							
		500 bp		500 bp							
		480 bp		400 bp							
		460 bp		300 bp							
		440 bp		200 bp							
		420 bp		100 bp							
		400 bp									
		380 bp									
		360 bp									
		340 bp									
		320 bp									
		300 bp									
		280 bp									
		260 bp									
		240 bp									

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Detection and Sizing of DNA in Agarose Gels

Continued

	20 bp	20 bp Ext	100 bp	100 bp ext	Tandem	500 bp	Quant Ladder	Rev Quant Ladder	50 bp – 1000 bp	50 bp – 2500 bp	1 kb – 10 kb
		220 bp									
		200 bp									
		180 bp									
		160 bp									
		140 bp									
		120 bp									
		100 bp									
		80 bp									
		60 bp									
		40 bp									
		20 bp									

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Detecting DNA with GelStar®, SYBR® Green I or II Nucleic Acid Gel Stains

Follow the Steps Below to Stain DNA after Electrophoresis

1. Remove the concentrated stock solution of GelStar® or SYBR® Green Stain from the freezer and allow the solution to thaw at room temperature.
2. Spin the solution in a microcentrifuge to collect the dye at the bottom of the tube.
3. Dilute the 10,000X concentrate to a 1X working solution (1 µL per 10 mL) in a pH 7.5–8.5 buffer - in a clear plastic polypropylene container. Prepare enough staining solution to just cover the top of the gel.
4. Remove the gel from the electrophoresis chamber.
5. Place the gel in staining solution.
6. Gently agitate the gel at room temperature.
7. Stain the gel for 15–30 minutes. The optimal staining time depends on the thickness of the gel, concentration of the agarose, and the fragment size to be detected. Longer staining times are required as gel thickness and agarose concentration increase.
8. Remove the gel from the staining solution and view with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® and SYBR® Green Stained Gels do not require destaining. The dyes' fluorescence yield is much greater when bound to DNA than when in solution.

Follow this Procedure When Including GelStar® Stain in the Agarose Gel

1. Remove the concentrated stock solution of GelStar® Stain from the freezer and allow the solution to thaw.
2. Spin the solution in a microcentrifuge tube.
3. Prepare the agarose solution (see pages 458–460).
4. Once the agarose solution has cooled to 70°C, add the stain by diluting the stock 1:10,000 into the gel solution prior to pouring the gel (1 µL per 10 mL).
5. Slowly swirl the solution.
6. Pour the gel into the casting tray (see page 460).
7. Load your DNA onto the gel.
8. Run the gel.
9. Remove the gel from the electrophoresis chamber.
10. View with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.). GelStar® Stained gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.

Staining Vertical Gels with GelStar® and SYBR® Green Stains

Incorporating GelStar® and SYBR® Green Stains into the gel or prestaining the DNA for use in a vertical format is not recommended. The dye binds to glass or plastic plates and DNA may show little to no signal. Gels should be post-stained as described in the previous section.

Follow this procedure when staining vertical gels with GelStar® or SYBR® Green Stain:

Follow steps 1–4, see above.

5. Open the cassette and leave the gel in place on one plate.
6. Place the plate, gel side up,in a staining container.
7. Gently pour the stain over the surface of the gel.
8. Stain the gel for 5–15 minutes.
9. View with a 300 nm UV transilluminator, CCD camera or Dark Reader® Transilluminator (Clare Chemical Research, Inc.) GelStar® or SYBR® Green Stained gels do not require destaining. The dye's fluorescence yield is much greater when bound to DNA than when in solution.

Detecting DNA with GelStar®, SYBR® Green I or II Nucleic Acid Gel Stains

Continued

Visualization by Photography

Gels stained with GelStar® and SYBR® Green Stains exhibit negligible background fluorescence, allowing long film exposures when detecting small amounts of DNA. Use the appropriate photographic filter for the stain.

Suggested film types and photographic conditions:

Polaroid® Film	f-stop	Exposure Time
Type 57 or 667	4.5	0.5–2 seconds
Type 55	4.5	15–45 seconds

Visualization by Image Capture System

For the best results and optimal sensitivity, visualize GelStar® Stained Gels on The Dark Reader® Transilluminator (Clare Chemical Research, Inc.) GelStar® and SYBR® Green Stains are compatible with most CCD and video imaging systems. Due to variations in the filters for these systems, you may need to purchase a new filter. Contact your system's manufacturer.

Stain	Emission (nm)	Excitation (nm)
GelStar® Stain	527	493
SYBR® Green I Stain	521	494
SYBR® Green II Stain	513	497

Application Notes

- The fluorescent characteristics of GelStar® and SYBR® Green Stains make them compatible with argon ion lasers.
- These stains are removed from double-stranded DNA by standard procedures for ethanol precipitation of nucleic acids.
- Gels previously stained with ethidium bromide can subsequently be stained with GelStar® or SYBR® Green Stain following the standard protocol for post-staining.
- The inclusion of GelStar® and SYBR® Green Stains in cesium chloride density gradient plasmid preparations is not recommended. The effect of the dye on the buoyant density of DNA is unknown.
- These stains do not appear to interfere with enzymatic reactions.
- We recommend the addition of 0.1% to 0.3% SDS in the prehybridization and hybridization solutions when performing Southern blots on gels stained with these dyes.
- Double-stranded DNA-bound GelStar® or SYBR® Green Stain fluoresces green under UV transillumination. Gels that contain DNA with single-stranded regions may fluoresce orange rather than green.

Decontamination

Staining solutions should be disposed of by passing through activated charcoal followed by incineration of the charcoal. For absorption on activated charcoal, consult Sambrook, *et al.*, pp. 6.16–6.19, (1989). Follow state and local guidelines for decontamination and disposal of nucleic acid staining solutions.

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Detecting DNA with Ethidium Bromide

Ethidium bromide is a fluorescent dye which detects both single- and double-stranded DNA. However, the affinity for single-stranded DNA is relatively low compared to double-stranded DNA. Ethidium bromide-stained DNA is detected by ultraviolet radiation. At 254 nm, UV light is absorbed by the DNA and transmitted to the dye; at 302 nm, and 366 nm, UV light is absorbed by the bound dye itself. In both cases, the energy is re-emitted at 590 nm in the red-orange region of the visible spectrum.

Procedure

For optimal resolution, sharpest bands and lowest background, stain the gel with ethidium bromide following electrophoresis.

Ethidium bromide can also be included in the gel and electrophoresis buffer (0.5 µg/mL) with only a minor loss of resolution. The electrophoretic mobility of DNA will be reduced by approximately 15%.

Follow the Steps below to Stain DNA After Electrophoresis

1. Prepare enough working solution of ethidium bromide. (0.5–1 µg/mL of ethidium bromide in distilled water or gel buffer) to cover the surface of the agarose gel.
2. Remove the gel from the electrophoresis chamber.
3. Submerge the gel for 20 minutes in the ethidium bromide solution.
4. Remove the gel from the solution.
5. Submerge the gel for 20 minutes in a new container filled with distilled water.
6. Repeat in fresh distilled water.
7. Gels can be viewed with a hand-held or tabletop UV light. For gel concentrations of 4% or greater, these times may need to be doubled. If after destaining the background is still too high, continue to destain.

Materials

- Staining vessel larger than gel
- UV transilluminator
- Magnetic stir plate
- Magnetic stir bar

Reagents

- Ethidium bromide stock solution (10 mg/mL)
- Electrophoresis buffer or distilled water

⚠ Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 474 before beginning these procedures.

Follow the Steps Below When Including Ethidium Bromide in the Agarose Gel

8. Prepare agarose solution (see pages 458–460).
9. While the agarose solution is cooling, add ethidium bromide to a final concentration of 0.1 to 0.5 µg/mL to the solution.
10. Gently swirl the solution.
11. Pour the gel into the casting tray.
12. Add ethidium bromide to the running buffer to a final concentration of 0.5 µg/mL.
13. Load and run the gel.
14. Destain the gel by submerging the gel in distilled water for 20 minutes.
15. Repeat in fresh distilled water.
16. Gels can be viewed with a hand-held or tabletop UV light during or after electrophoresis.

Decontamination of Ethidium Bromide Solutions

Decontamination of ethidium bromide solutions is described in Sambrook, *et al.*, pp. 6.16–6.17 (1989).

Follow local guidelines and regulations for ethidium bromide decontamination and disposal.

Recovery of DNA from Agarose Gels

Tips for Increasing DNA Recovery Efficiency from Agarose Gels

This section discusses various tips which will increase the efficiency of recovery of DNA from agarose gels in all recovery techniques.

Choosing the Appropriate Agarose for Recovery

When recovering DNA, the choice of agarose is one of the most important factors. To avoid recovery altogether, one can choose to perform in-gel reactions.

We offer Genetic Technology Grade™ (GTG™) Products that are specially prepared for demanding molecular biology applications. See pages 281–293. (See table below for our agaroses and compatible recovery techniques.)

Buffer Types

When recovering DNA from agarose gels, 1X Tris-acetate (TAE) Buffer is recommended for electrophoresis.

Casting and DNA Loading Tips

- Prepare the gel in 1X TAE Buffer
- Do not cast the gel with ethidium bromide
- Cast a gel 3–4 mm thick
- Use a comb ≤1 mm thick
- Load no more than 100 ng of DNA per band

Staining and Recovery Tips

When recovering DNA from agarose gels, we recommend the following:

- Stain the gel for 15–20 minutes
- Destain the gel in distilled water for two, 20-minute washes
- Do not expose the DNA to UV light for any longer than 1 minute; long exposure of DNA to UV light can nick the DNA
- The addition of 1 mM guanosine or cytidine to the gel and electrophoresis buffer is effective in protecting DNA against UV-induced damage
- Cut the smallest gel slice possible

It is possible to avoid staining samples which will be used for recovery by running an additional lane containing a small amount of your sample immediately next to the molecular weight marker. However, DNA is damaged by UV light in the absence of ethidium bromide so keep exposure to UV light as brief as possible. Cut the lanes containing the marker and the small amount of the sample from the rest of the gel and stain. To recover the preparative loading, line up the stained portion of the gel with the unstained portion. Check by placing on UV transilluminator and cut out the area that lines up with your sample on the unstained portion of the gel.

The FlashGel™ System for Recovery, (page 296–297) offers a non-UV alternative for DNA recovery.

Reference

Grundemann, D. and Schomig, E., BioTechniques 21(5): 898–903, 1996.

Lonza Agaroses and Compatible Recovery Techniques

	In-Gel	β-Agarase	Phenol/Chloroform	Recovery Columns	Electroelution	Freeze/Squeeze
SeaKem® GTG™ Agarose			■	■	■	■
SeaPlaque™ GTG™ Agarose	■	■	■	■	■	■
NuSieve™ GTG™ Agarose	■	■	■	■	■	■
MetaPhor™ Agarose			■	■	■	■
SeaPlaque™ Agarose		■	■	■	■	■

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Phenol/Chloroform Extraction of DNA from Agarose Gels

Compatible Agaroses

- SeaPlaque™ GTG™ Agarose (certified and tested for the recovery of DNA)
- NuSieve™ GTG™ Agarose (certified and tested for the recovery of DNA)
- SeaPlaque™ Agarose

Tips

Recovery failures when extracting DNA from agarose using phenol/chloroform most often result from either extracting too large a piece of agarose, or precipitating agarose along with the DNA at the ethanol precipitation step. To address these difficulties, we recommend the following:

- No more than 200 mg (200 µL) of agarose should be extracted in a single tube; if your gel slice containing the DNA is larger than this, separate it into smaller pieces, then combine the extracted solutions prior to ethanol precipitation
- Ethanol precipitation of agarose can be avoided by chilling the extracted solution on ice for 15 minutes, then centrifuging the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge **prior to adding salts and ethanol**. The supernatant is then carefully decanted, and the DNA in the supernatant is precipitated following standard protocols
- Not useful for large DNA (>10kb). Vortexing will shear the DNA

Ethanol Precipitation of DNA Recovered from Agarose Gels

Tips

- Prior to adding salts and ethanol, precipitation of agarose can be avoided by chilling the supernatant on ice for 15 minutes, then centrifuging the sample(s) in a cold room for 15 minutes at maximum speed in a microcentrifuge. The supernatant is then carefully decanted, and the DNA in the supernatant is ethanol precipitated following standard protocols
- Ethanol precipitations should be incubated at room temperature with ammonium acetate rather than sodium acetate in order to decrease the likelihood of coprecipitation of agarose-oligosaccharides with the DNA or RNA

Buffers for Protein Electrophoresis

The Laemmli Buffer System (Tris-Glycine) is a discontinuous buffer system, widely used for fine resolution of a broad molecular weight range of proteins. In this system, the gel is prepared with Tris-HCl Buffer and the Tris-glycine is used as the running buffer.

In the Tris-Tricine Buffer System, tricine replaces glycine in the running buffer. The result is more efficient stacking and destacking, and higher resolution of proteins and peptides with lower molecular weights (under 10 kDa – 15 kDa). For fast reliable separation in Laemmli gels such as PAGEr™ Gold precast gels or when using PAGEr™ EX precast gels use ProSieve™ EX Running Buffer. See page 325.

Buffer Preparation Tris-Glycine SDS Buffer, pH 8.3	
10x Stock solution	g/L for 10X Stock solution
0.25 M Tris base	30.3 g Tris Base
1.92 M Glycine	144.0 g Glycine
1.0% SDS*	Adjust volume to 1 liter with distilled water

(1X = 25 mM Tris base, 192 mM Glycine, 0.1% SDS*)
*Omit SDS if running native proteins.

Tris-Tricine SDS Buffer, pH 8.3	
10x Stock solution	g/L for 10X Stock solution
1 M Tris base	121.1 g Tris base
1 M Tricine	179.0 g Tricine
1.0% SDS*	Adjust volume to 1 liter with distilled water

(1X = 100 mM Tris base, 100 mM Tricine, 0.1% SDS*)
*Omit SDS if running native proteins.

2X Tris-Glycine SDS Sample Buffer	
2X concentrate	amount to add for 2X concentrate
126 mM Tris-HCl, pH 6.8	2.5 mL of 0.5 M Tris-HCl, pH 6.8
20% Glycerol	2 mL Glycerol
4% SDS	4 mL of 10% SDS
0.005% Bromophenol blue	0.5 mL of 0.1% Bromophenol blue
Adjust volume to 10 mL with distilled water	

(1X = 63 mM Tris-HCl, 10% glycerol, 2% SDS, 0.0025% Bromophenol blue, 2.5% βME)

Loading and Running Proteins on Polyacrylamide Gels

Protein load levels will vary depending upon sample purity and staining method used. For highly purified proteins, 0.5 µg to 5 µg protein per lane on a minigel is generally sufficient. Complete mixtures such as cell lysates may require as much as 50 µg protein per lane. The table below provides lower detection limits for protein detection.

Protein Stain Detection Limits

Protein stain	Lower Detection Limit (protein/band)
Coomassie® Blue Stain	100 ng
Silver Stain	1 ng
SYPRO® Orange Protein Gel Stain	1 ng–2 ng
SYPRO® Red Protein Gel Stain	1 ng–2 ng
SYPRO® Tangerine Protein Gel Stain	4 ng–8 ng
ProSieve™ Safe Stain	8 ng–15 ng

NOTE: Limits are based on optimal detection methods for each stain.

Optimal Voltage and Power Settings

Tris-glycine polyacrylamide minigels are typically run at constant voltage between 125–200 volts. During electrophoresis, the current drops and heat decreases. If the voltage is set too high or not limited, excessive heat is produced resulting in band distortion and potential damage to the gel and apparatus. Constant voltage allows the same voltage to be used with multiple gels in an apparatus. Gel thickness is not a factor when using constant voltage. For large format gels, a constant current setting with a voltage limit set slightly higher (5 volts) than the expected voltage for the run may also be used to maintain sample velocity.

By substituting 1X ProSieve™ EX Running Buffer for Tris-glycine SDS one can run polyacrylamide mini-gels at a higher voltage of 200–250V for much faster runs while maintaining resolution of bands. See page 325.

Using PAGEr™ EX Gels and 1X ProSieve™ EX Running Buffer maximizes speed and resolution. See page 323.

Optimal Electrophoretic Time

The gel should be run until the bromophenol blue dye has migrated to the bottom of the gel. Gel running times are dependent upon the buffer system used, the length of the gel and the polyacrylamide concentration. Typically minigels will take approximately 30–90 minutes to run, whereas large format gels may take as long as five hours to run.

By substituting 1X ProSieve™ EX Running Buffer for Tris-Glycine SDS and running at a higher voltage of 200–250V the time can be cut to as little as 15 minutes while maintaining resolution of bands. See page 323.

Using PAGEr™ EX Gels and 1X ProSieve™ EX Running Buffer maximizes speed and resolution. See page 323.

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Blotting Proteins from Polyacrylamide Gels

Introduction

Protein transfer efficiency in blotting applications is dependent upon multiple factors, including gel percentage, gel thickness, protein size, transfer conditions (e.g., buffer and voltage), and type and quality of membrane. To achieve optimal transfer efficiency, transfer conditions must be adjusted to address these varying factors.

Choosing the Appropriate Membrane

Nitrocellulose	PVDF	Nylon
Hydrophobic binding	Hydrophobic binding	Hydrophobic and electrostatic binding
General purpose membrane	SDS tolerant	Stable if baked
Low background	High background	High background
Low strength	High strength	High strength
Becomes brittle if baked	Suitable for protein sequencing	Least suitable for Western transfer

Transfer Solutions

Formula for Towbin Transfer Solution

1X Working Solution	Amount for 1X Working Solution
25 mM Tris base	30.3 g Tris base
192 mM Glycine	144.1 g Glycine
0.1% SDS	10.0 g
	Adjust volume to 8 liters with distilled water. Measure, but do not adjust pH; it should be approximately 8.2 to 8.4
20% Methanol	2 L Methanol Adjust volume to 10 liters with distilled water

It may be necessary to lower the concentrations of methanol, SDS or both to obtain the optimal balance of transfer and binding efficiency. The table below outlines the effects that SDS and methanol have on protein transfer.

SDS	Methanol
Improves transfer of proteins >60 kDa	Improves binding efficiency
Decreases binding efficiency	Decreases transfer efficiency
Not compatible with nylon membranes	Do not soak gel in transfer buffer prior to blotting
Include 0.1%–0.2% in transfer buffer	Include 20% in transfer buffer

For rapid efficient transfer of proteins from PAGEr™ Gold and PAGEr™ EX Gels or other Laemmli precast mini gels use 1X ProSieve™ EX Western Transfer Buffer. It adds speed, is easy to use and does not contain methanol. See page 325.

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Electrophoretic Theory

Electrophoretic Parameters

During electrophoresis, one of the parameters is held constant and the other two are allowed to vary as the resistance of the electrophoretic system changes. In vertical systems, the resistance of the gel increases as highly conductive ions like Cl are electrophoresed out of the gel. As these ions are removed from the gel, the current is carried by less conductive ions like glycine, borate, acetate, etc. Under normal conditions in horizontal systems, there is little change in resistance. However, with high voltage or extended runs in horizontal systems, resistance can decrease.

Introduction

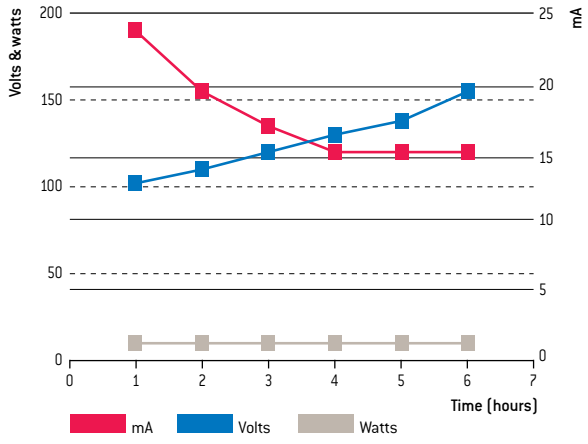
There are advantages and disadvantages for setting each of the critical parameters as the limiting factor in electrophoresis. Sequencing gels are usually run at constant wattage to maintain a uniform temperature. Agarose and acrylamide gels for protein and DNA separation are run at constant voltage or constant current.

Constant Wattage

In a vertical system when wattage is held constant, the velocity of the samples will decrease because the current, which is in part carried by the DNA, decreases to compensate for the increase in voltage. The generation of heat will remain uniform.

If the current should decrease disproportionately (from a buffer problem, a buffer leak or a hardware problem), the power supply will increase the voltage to compensate.

Since voltage and current vary over time at a constant wattage, it is not possible to predict mobility of samples from the calculation of watt-hours.



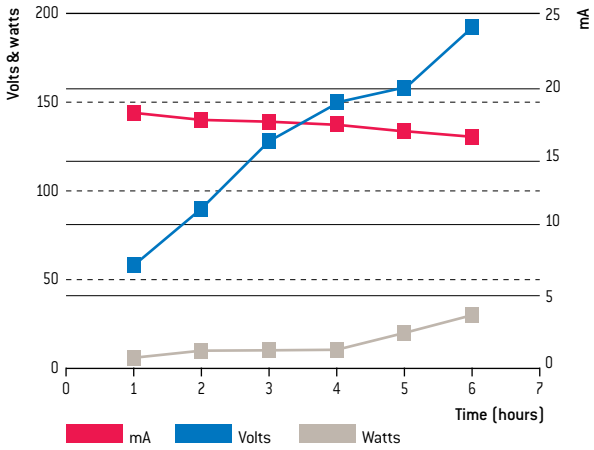
Reference

Rickwood, D. and Hames, B.D., Gel Electrophoresis of Nucleic Acids: A Practical Approach, IRL Press Limited, 1982.

Constant Current

When the current is held constant, the samples will migrate at a constant rate. Voltage and wattage will increase as the resistance increases, resulting in an increase in heat generation during the run.

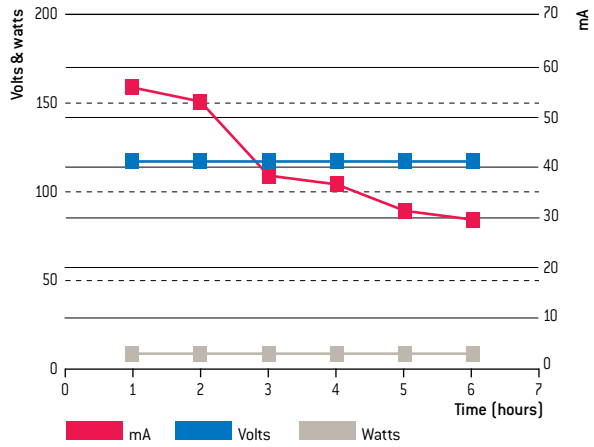
If a break occurs in the system, such as a damaged lead or electrode or a buffer leak, the resistance of the gel will be greatly increased. This will cause a large increase in wattage and voltage resulting in the generation of excessive heat. It is even possible for the system to get hot enough to boil, or start the apparatus to scorch or burn.



Constant Voltage

When voltage is set constant, current and wattage will decrease as the resistance increases, resulting in a decrease of heat and DNA migration.

Since the heat generated will decrease, the margin of safety will increase over the length of the run. If a problem develops and the resistance increases dramatically, the current and wattage will fall since the voltage cannot increase. Even if the apparatus fails, the worst that is likely to happen is that the resistance will increase so much that the power supply will not be able to compensate, and it will shut off.



Safety and Environmental Precautions

In general, working with nucleic acids and proteins does not present significant hazards to humans so long as precautions are taken to protect against certain harmful materials.

Throughout the Technical Information section are references to materials and methods which are hazardous to humans and the environment. Specific hazards and protection steps are summarized here, and it is recommended that trained users follow these precautions when performing the operations outlined in this manual.

These precautions are not a substitute for proper health and safety training, nor are they a substitute for the user institution's standards and procedures, or local governmental requirements. In each case, the user should be aware of and follow local guidelines for handling and disposal of these materials.

Specific Chemical Hazards

Ethidium Bromide

Ethidium bromide (EtBr) is a known mutagen and a suspected carcinogen. Care should be taken to prevent exposure. Recommended personal protective equipment includes: nitrile gloves, lab coats, and safety glasses. Use ethidium bromide solutions in a well ventilated area, and prevent inhalation of vapors. Electrophoresis tanks should be kept covered during electrophoresis of gels containing ethidium bromide. Ethidium bromide powder should be handled in a fume hood.

Decontamination and disposal of ethidium bromide should be done according to local government and institutional regulations.

GelStar® and SYBR® Green Nucleic Acid Gel Stains

GelStar® and SYBR® Green Stains contain a component which can penetrate cells (including skin), and is a potential mutagen. Therefore, care should be taken to prevent exposure. Recommended personal protective equipment includes: (non nitrile) gloves, lab coats, and safety glasses.

Decontamination and disposal of these stains should be done according to local government and institutional regulations.

Formaldehyde and Formamide

Formaldehyde and formamide are known carcinogens, and exposure should be limited to as low as feasible. Gloves, safety glasses (or goggles when pouring liquid), and lab coats should be worn. Operations using formaldehyde or formamide should be conducted with the use of respiratory protection, or in a fume hood or other well ventilated area to prevent inhalation.

Disposal of these materials should be done according to local government and institutional regulations.

DMSO

Dimethyl Sulfoxide (DMSO) can penetrate cells and DNA, and is a carrier of other substances in solutions into those cells. Care should be taken to prevent exposure, including the use of non nitrile gloves (natural rubber are recommended), safety glasses and lab coat.

Disposal of these materials should be done according to local government and institutional regulations.

Phenol

Phenol is toxic by inhalation, ingestion, and contact. It will burn eyes. Appropriate gloves, safety glasses (or goggles), and lab coats are required. Proper ventilation should be utilized.

Disposal of these materials should be done according to local government and institutional regulations.

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