



SZABO SCANDIC

Part of Europa Biosite

Produktinformation



Forschungsprodukte & Biochemikalien



Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere [Liefer- und Versandbedingungen](#)

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC Handels GmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

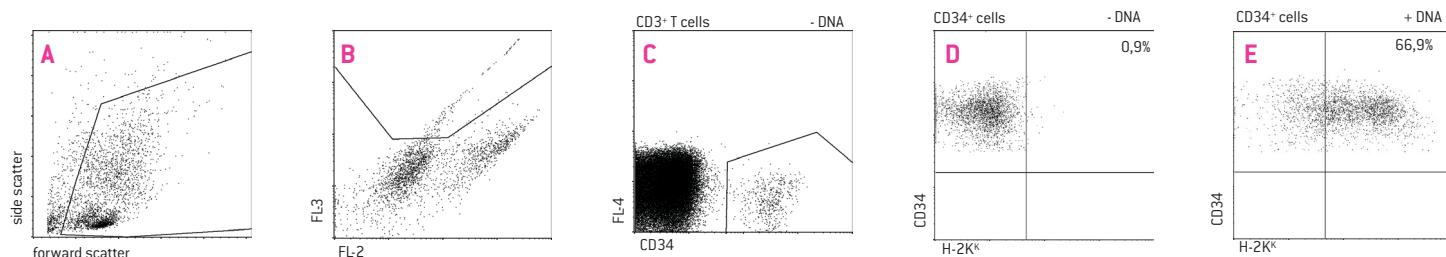
[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Amaxa® Human CD34⁺ Cell Nucleofector® Kit

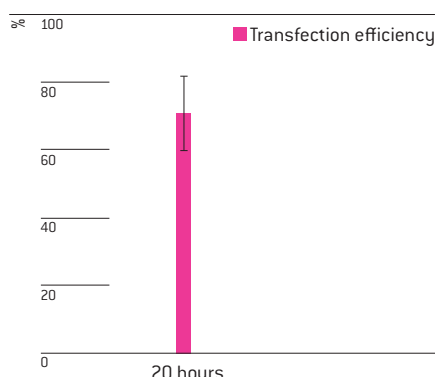
For Human CD34⁺ Cells

Unstimulated human CD34⁺ cells (small round lymphoblastoid cells) represent a subpopulation of mononuclear cells from peripheral blood mononuclear cells (PBMC), from leukapheresis products after hematopoietic progenitor cell mobilization or from cord blood.

Example for Nucleofection® of CD34⁺ hematopoietic progenitor cells with H-2K^k cDNA



Fresh peripheral blood mononuclear cells (PBMC) partially enriched for CD34⁺ cells were transfected by Nucleofection® using the Human CD34⁺ Cell Nucleofector® Kit and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K^k. 16 hours post Nucleofection®, the cells were stained with an APC-coupled antibody directed against CD34⁺, a PE-coupled antibody directed against H-2K^k and were analyzed by flow cytometry. Lymphocytes were gated according to forward/side scatter (A). Dead cells and CD34⁺ cells were excluded by staining with propidium iodide and gating (B, C). H-2K^k expression is shown post Nucleofection® without (D) and with plasmid DNA (E).



Transfection efficiencies of fresh, un-stimulated human CD34⁺ cells (partially enriched) 20 hours post Nucleofection®. Cells were transfected by Nucleofection® with program U-008 and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K^k.

Product Description

Cat. No.	VPA-1003
Size (Reactions)	25
Human CD34 ⁺ Cell Nucleofector® Solution	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified Cuvettes	25
Plastic Pipettes	25
Storage and stability	Store Nucleofector® Solution, Supplement and pmaxGFP® Vector at 4°C. For long-term storage, pmaxGFP® Vector is ideally stored at -20°C. The expiration date is printed on the solution box. Once the Nucleofector® Supplement is added to the Nucleofector® Solution it is stable for three months at 4°C.

Required Material

Note Please make sure that the entire supplement is added to the Nucleofector® Solution. The ratio of Nucleofector® Solution to supplement is 4.5 : 1. For a single reaction use 82 µl of Nucleofector® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.

- Nucleofector® Device
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- 12-well culture dish or culture system of choice
- **Culture medium:** RPMI 1640 [Lonza; Cat. No. 12-167F] supplemented with 10% autologous serum or 10% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, and 2 mM UltraGlutamine I [Lonza, Cat. no. BE17-605E/U1]
- **Stimulation medium** (optional; post Nucleofection): Culture medium supplemented e.g. with 50 ng/ml SCF [Promocell; Cat. No. C-63120], 10 ng/ml IL-3 [Promocell; Cat. No. C-61320], and 20 ng/ml IL-6 [Promocell; Cat. No. C-61630]
- PBS containing 0.5% BSA (PBS/BSA)
- Ficoll-Paque™ Plus [GE Healthcare; Cat. No. 17-1440-03]
- Prewarm appropriate volume of culture media (2 ml per reaction) to 37°C
- Appropriate number of cells (1 – 5 x 10⁶ cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Blood samples

- 1.1 Fresh human blood treated with an anticoagulant (e.g. heparin, citrate, ACD-A) or alternatively, leukocyte-enriched buffy coat not older than 8 hours. The samples should be diluted with 2 – 4 volumes PBS/BSA

Preparation of PBMC

- 1.2 Pipet 15 ml Ficoll-Paque™ Plus in a 50 ml conical tube
- 1.3 Overlay Ficoll- Paque™ Plus with 35 ml blood sample and centrifuge at 750xg for 20 minutes at 20°C in a swinging-bucket rotor without brake
- 1.4 Remove the upper layer leaving the mononuclear cell layer undisturbed at the interphase. Carefully transfer the interphase cells (lymphocytes and monocytes) to a new 50 ml conical tube
- 1.5 Add PBS/BSA to 50 ml mark, mix and centrifuge at 350xg for 10 minutes at 4°C. Remove the supernatant carefully
- 1.6 Resuspend the cell pellet in 25 ml of PBS/BSA and centrifuge at 160xg for 15 minutes at 4°C. Remove the supernatant carefully
- 1.7 Resuspend the cell pellet in 25 ml PBS/BSA and centrifuge at 300xg for 10 min at 4°C. Remove the supernatant carefully
- 1.8 Resuspend cell pellet in 5 ml PBS/BSA and count the cells

Preparation of CD34⁺ cells before Nucleofection®

- 1.9 It is preferable to use freshly isolated PBMC or fresh CD34⁺ (human hematopoietic progenitor) cell enriched preparations (e.g. by magnetic separation) for Nucleofection®
- 1.10 Purified CD34⁺ cells (e.g. from leukapheresis) may be frozen for longterm storage. Frozen CD34⁺ cells should be thawed and cultured for 1 – 2 hours prior to Nucleofection®

Note Purification of CD34⁺ cells from frozen leukapheresis is not advisable.

2. Nucleofection®

One Nucleofection® Sample contains

1 – 5 x 10 ⁶ cells
1 – 5 µg plasmid DNA (in 1 – 5 µl H ₂ O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 – 30 pmol/sample)
100 µl Human CD34 ⁺ Cell Nucleofector® Solution

- 2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
- 2.2 Prepare 12-well plates by filling appropriate number of wells with 1.5 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO₂ incubator
- 2.3 Count the cells and determine cell density
- 2.4 Centrifuge the required numbers of cells (1 – 5 x 10⁶ cells per sample) at 200xg for 10 minutes at room temperature. Discard supernatant completely so that no residual PBS/BSA covers the cell pellet
- 2.5 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample. Avoid storing the cell suspension longer than 20 minutes in Human CD34⁺ Cell Nucleofector® Solution, as this reduces cell viability and gene transfer efficiency
- 2.6 Combine 100 µl of cell suspension with 1 – 5 µg DNA or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 - 30 pmol/sample) or other substrates
- 2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
- 2.8 Select the appropriate Nucleofector® Program U-008 (U-08 for Nucleofector® I Device)
- 2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
- 2.10 Take the cuvette out of the holder once the program is finished
- 2.11 Add ~500 µl of the pre-equilibrated supplemented culture medium to the cuvette and gently transfer the sample into the 12-well plate (final volume of 2 ml media per well/sample). Use the supplied pipettes and avoid repeated aspiration of the sample

3. Post Nucleofection®

- 3.1 Incubate the cells in humidified 37°C/5% CO₂ incubator until analysis. Gene expression is often detectable after only 4 – 8 hours. If this is not the case, incubation period may be prolonged. If necessary, cells can be cultivated and stimulated post Nucleofection® in stimulation media (seeding conditions: 0.5 – 2 x 10⁶ cells per ml; replace medium every 2 – 3 days)

Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
www.lonza.com/nucleofection-citations

For more technical assistance, contact our Scientific Support Team:

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: scientific.support@lonza.com

Europe and Rest of World
Phone: +49 221 99199 400
Fax: +49 221 99199 499
E-mail: scientific.support.eu@lonza.com

Lonza Cologne AG
50829 Cologne, Germany

Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans. The Nucleofector® Technology, comprising Nucleofection® Process, Nucleofector® Device, Nucleofector® Solutions, Nucleofector® 96-well Shuttle® System and 96-well Nucleocuvette® plates and modules is covered by patent and/or patent-pending rights owned by Lonza Cologne AG.

Amaxa, Nucleofector, Nucleofection and maxGFP are either registered trademarks or trademarks of the Lonza Cologne AG in Germany and/or U.S. and/or other countries.

Ficoll-Paque is a trademark of GE Healthcare.

Other product and company names mentioned herein are the trademarks of their respective owners.

This kit contains a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as a positive control with this Lonza product only. Any use of the proprietary nucleic acid or protein other than as a positive control with this Lonza product is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at license@evrogen.com.

The CMV promoter is covered under U.S. Patents 5,168,062 and 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 52242.

The use of this product in conjunction with materials or methods of third parties may require a license by a third party. User shall be fully responsible for determining whether and from which third party it requires such license and for the obtainment of such license.

No statement is intended or should be construed as a recommendation to infringe any existing patent.

© Copyright 2009, Lonza Cologne AG. All rights reserved DPA-1003 08/09