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Produktinformation

- Forschungsprodukte & Biochemikalien
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- 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 HandelsgmbH

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

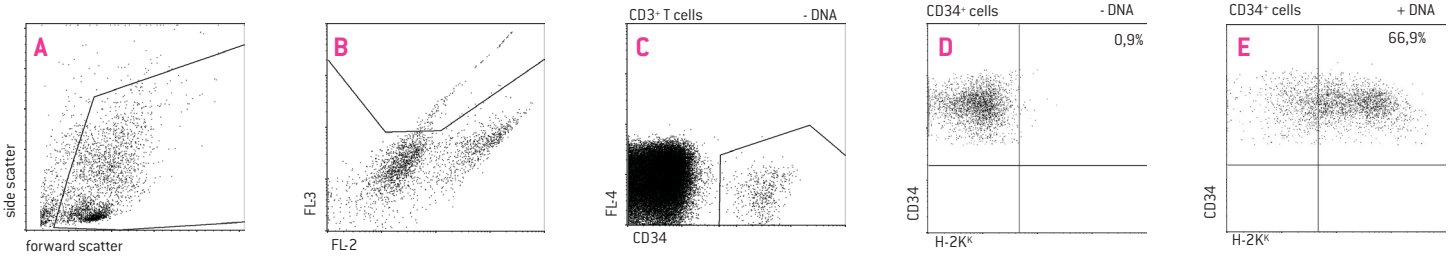
linkedin.com/company/szaboscandic

Amaxa® Human CD34+ Cell Nucleofector® Kit

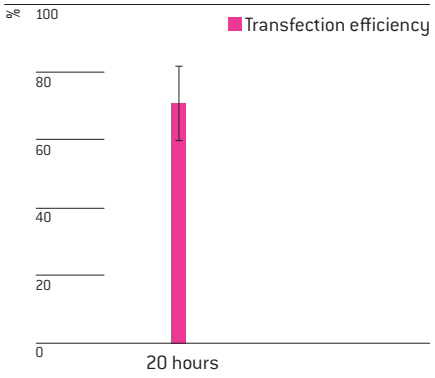
For Human CD34+ Cells

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-2Kk 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-2Kk. 16 hours post Nucleofection®, the cells were stained with an APC-coupled antibody directed against CD34+, a PE-coupled antibody directed against H-2Kk 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-2Kk 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-2Kk.

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 10^6 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_2O 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% CO_2 incubator

2.3 Count the cells and determine cell density

2.4 Centrifuge the required numbers of cells (**1 – 5 x 10^6 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% CO_2 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 10^6 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

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