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- Trockeneiszuschlag
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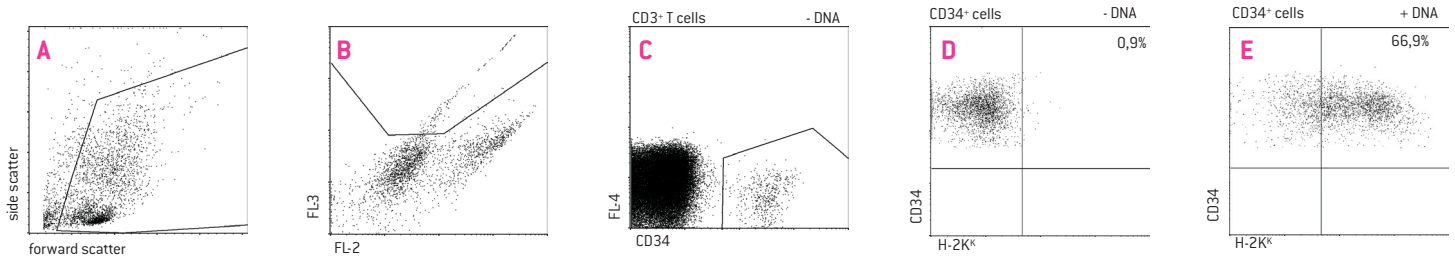
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Amaxa[®] Human CD34⁺ Cell Nucleofector[®] Kit

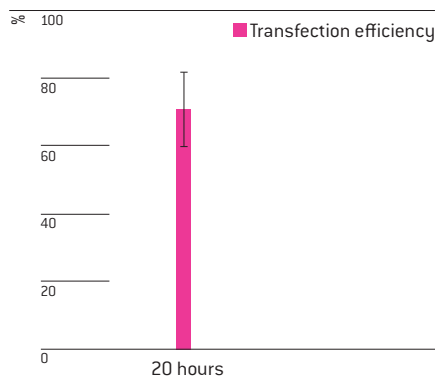
For Human CD34⁺ Cells

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 10⁶ cells

1 – 5 µg plasmid DNA (in 1 – 5 µl H₂O 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:

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