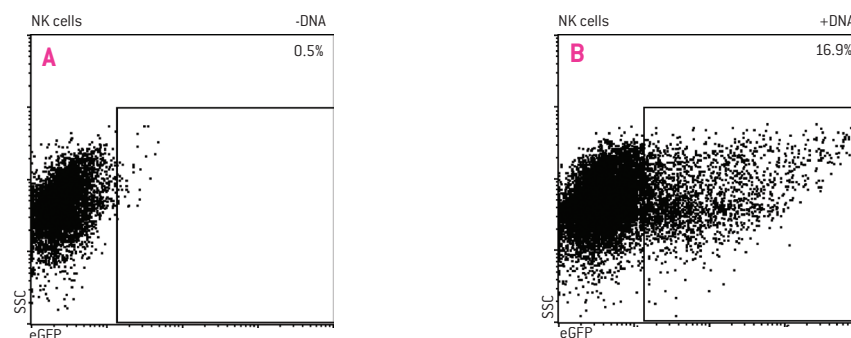


Amaxa[®] Human NK Cell Nucleofector[®] Kit

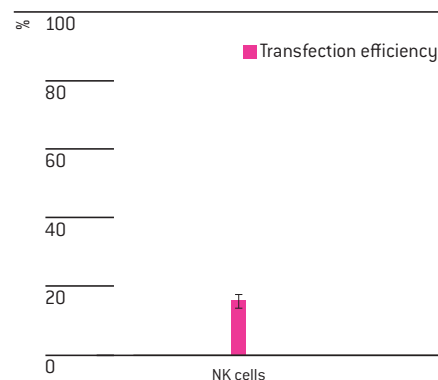
For human Natural Killer Cells (NK Cells)

CD56⁺/CD3⁻ human NK cells (small round lymphoblastoid cells) are a sub-population of human peripheral blood mononuclear cells (PBMC). This protocol is designed for freshly isolated primary human natural killer cells.

Example for Nucleofection[®] of primary human NK cells with eGFP cDNA



Polyclonal human NK cells generated from PBMC co-cultured with the feeder cell line RPMI 8866 for 9 days were transfected with the Human NK Cell Nucleofector[®] Kit and a plasmid encoding eGFP. 24 hours post transfection cells were stained with propidium iodide (PI) to exclude dead cells and analyzed by flow cytometry for GFP expression. The GFP expression of NK cells is shown after Nucleofection[®] with program U-001 without (A) and with DNA (B) [courtesy of J. Sundbäck and K. Kärre, Karolinska Institute, Microbiology and Tumor Biology Center, Stockholm, Sweden].



Transfection efficiency of primary human NK cells 24 hours post Nucleofection[®]. Cells were transfected by Nucleofection[®] with program U-001 and a plasmid encoding either the enhanced green fluorescent protein eGFP or the yellow fluorescent protein eYFP. Viability: 50 – 60%.

Product Description

Cat. No.	VPA-1005
Size (Reactions)	25
Human NK 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 your choice
- **Culture medium:** Clonetics® Lymphocyte Growth Media-3 LGM-3® for serum-free culture [Lonza, Cat.No. CC-3211] or RPMI 1640 [Lonza; Cat. No. 12-167F] with 10% fetal calf serum (FCS), each supplemented with 100 µg/ml streptomycin, 100 U/ml penicillin, 2 mM UltraGlutamine I [Lonza; Cat. No. BE17-605E/U1], 200 – 300 U/ml IL-2 and 10 U/ml IL-15
- **For isolation:** PBS containing 0.5% BSA (PBS/BSA); Ficoll-Paque™ Plus [GE Healthcare; Cat. No. 17-1440-03]
- **For enrichment:** CD56 Multisort Kit [Miltenyi Biotec; Cat.No. 130-090-755]
- Prewarm appropriate volume of culture media to 37°C [2 ml per sample]
- Appropriate number of cells (2 – 3 x 10⁶ cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Notes

- This protocol is designed for freshly isolated primary human natural killer cells.
- Transfection results may be donor-dependent.
- For preparation, do not perform protocols using hypo-osmolar buffers. This may lead to high cell mortality after Nucleofection®.
- For freshly isolated cells no cultivation is required prior to Nucleofection®. For cryopreserved cells we recommend incubating the thawed cells for 1 – 2 hours at 37°C in culture medium before 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 of PBS containing 0.5% BSA (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 minutes at 4°C. Remove the supernatant carefully
- 1.8 Resuspend cell pellet in 5 ml PBS/BSA and count the cells

Note Purified PBMC may be stored at 4°C overnight in PBS/BSA, but this can cause a significant loss of transfection efficiency.

Preparation of NK cells

- 1.9 Preferably, fresh CD56⁺/CD3⁻ NK cells enriched by magnetic separation (e.g. with CD56 Multisort Kit) should be used for Nucleofection®

2. Nucleofection®

One Nucleofection® Sample contains

2 – 3 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 NK 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 (2 – 3 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 NK 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 appropriate amount of siRNA 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-001 (U-01 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 culture media 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 should be analyzed at different time points e.g. after 6 – 24 hours

Additional Information

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

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