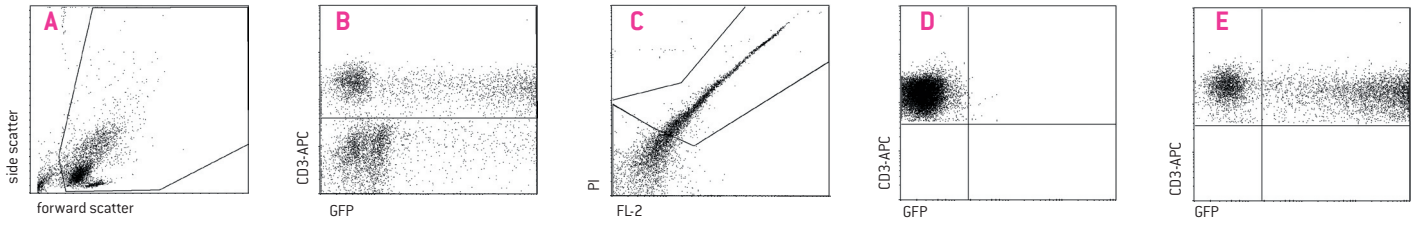
Amaxa® Human T Cell Nucleofector® Kit

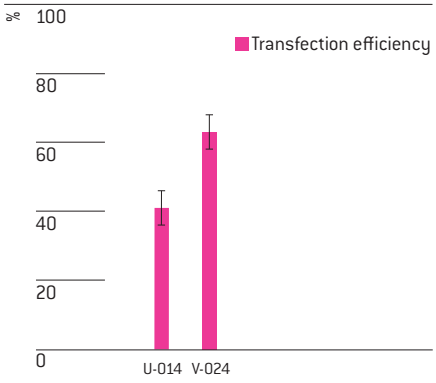
For unstimulated Human T Cells

Small round lymphoblastoid cells; subpopulation of human peripheral blood mononuclear cells (PBMC). PBMC should be purified from fresh human blood samples treated with an anti-coagulant or from leukocyte-enriched buffy coat

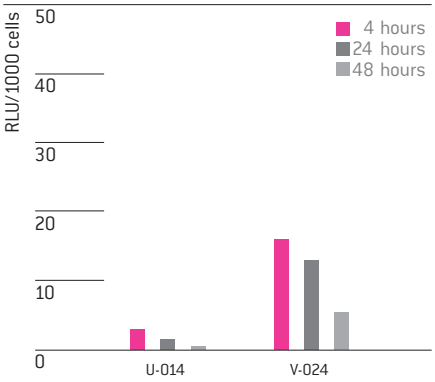
Example for Nucleofection® of CD3+ T cells with different reporter genes



PBMC were freshly isolated from buffy coat and transfected with Nucleofector® Program V-024 and 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® Protein E xpression of T cells is shown after Nucleofection® without (D) and with plasmid DNA (E).



Transfection efficiency of human T cells 24 hours post Nucleofection®. Cells were transfected with program U-014 or V-024 and 2 µg pmaxGFP® Vector. Cell viability (% PI negative) is usually 80% for both programs. However, the absolute number of viable cells is reduced with program V-024.



Luciferase expression of purified human T cells. Human T cells were purified by MACS separation. Purified T cells were transfected with the Human T Cell Nucleofector® Kit, program U-014 or program V-024 and 2 µg of a pGl3-CMV plasmid. Luciferase expression was analyzed 4 hours, 24 hours and 48 hours after Nucleofection® and RLU were normalized to 1000 cells.

Product Description

Cat. No.	**VPA-1002**
Size (reactions)	25
Human T 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 plate or 6-well culture plate for coating (see below) 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 BioWhittaker® IMDM media for addition of 10% serum [Lonza, Cat.No. BE12-722F]
- **For isolation:** Ficoll-Paque™ Plus [GE Healthcare; Cat. No. 17-1440-03]; PBS containing 0.5% [w/v] BSA (PBS/BSA)
- **For enrichment (optional):** Pan T Cell Isolation Kit II [Miltenyi Biotec; Cat. No. 130-091-156] or RosetteSep™ Isolation Kit for human T cells [StemCell Technologies, Cat. No 15021]
- **For coating of plates** (for post Nucleofection® stimulation): Anti-Human CD3 MAB [OKt 3; eBioscience, Cat. No. 14-0037-82] and Anti-Human CD28 MAB [5E8; Research Diagnostics Inc., Cat. No. 10R-CD28bHUµg/µl]; control antibody [purified mIgG(K); BD-Pharmingen, Cat. No. 554 721]; antibodies should be diluted in carbonate buffer (32 mM Na_2CO_3/16 mM $NaHCO_3$) from 100 ng/µl stock solutions directly before use; Immuno™ Plate C96 Maxi Sorp™ [Nunc, Cat. No.: 430 341]
- Prewarm appropriate volume of culture medium to 37°C (2 ml per sample)
- Appropriate number of cells (5 – 10 x 10^6 cells per sample; minimal cell number: 7 x 10^5 cells, a lower cell number may lead to a major decrease of cell viability; maximal cell number: 2 x 10^7 cells)

1. Pre Nucleofection®

Notes

- **This protocol is designed for fresh unstimulated primary human T cells from whole PBMCs. Depending on application T cells can be further enriched (see below)**
- **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®**
- **For Nucleofection® of stimulated T cells, please refer to the Optimized Protocol for stimulated Human T Cells**

Coating of culture plates (optional for stimulation post Nucleofection®)

1.1 Incubate each well with 1 ml (for 6-well) or 50 µl (for 96-well; Nunc Immuno™ Plate C96 Maxi Sorp™) of a solution of Anti-Human CD3 MAB at a final concentration of 1 µg/ml and Anti-Human CD28 MAB at a final concentration of 2 µg/ml (or with a solution of a control antibody (purified mIgG(K)) at a final concentration 3 ug/ml)at 37°C/5% CO_2 for 5 hours

1.2 Wash the wells carefully three times with PBS/BSA

Blood samples

1.3 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

1.4 Pipet 15 ml Ficoll-Paque™Plus in a 50 ml conical tube
1.5 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
1.6 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
1.7 Add PBS/BSA to 50 ml, mix and centrifuge at 350xg for 10 minutes at 4°C. Remove the supernatant carefully
1.8 Resuspend the cell pellet in 25 ml of PBS/BSA and centrifuge at 160xg for 15 minutes at 4°C. Remove the supernatant carefully
1.9 Resuspend the cell pellet in 25 ml PBS/BSA and centrifuge at 300xg for 10 minutes at 4°C. Remove the supernatant carefully
1.10 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.**

Enrichment of T cells (optional)

1.11 Primary human T cells can be further enriched by using Pan T Cell Isolation Kit II [Miltenyi] or RosetteSep™ Isolation Kit for human T cells [StemCell Technologies] according to the manufacturer's protocol

2. Nucleofection®

One Nucleofection® Sample contains
5 – 10 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 T 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 2 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator for at least 30 minutes
2.3 Count the cells and determine cell density
2.4 Centrifuge the required numbers of cells (5 – 10 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 T 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**, 2 µg pmaxGFP® Vector or **30 nM – 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-014** for high viability (e.g. T cells that should be stimulated post Nucleofection®) or **V-024** for high expression level (U-14 or V24 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 a humidified 37°C/5% CO_2 incubator until analysis. Gene expression is often detectable after only 4 – 8 hours
3.2 Unstimulated T cells can be cultured up to 48 hours. Medium change 6 hours post Nucleofection® leads to an increased viability after transfection. Spin the culture dishes for 8 minutes at 140xg in a tissue culture centrifuge. Carefully remove the medium and add fresh pre-equilibrated culture medium. Alternatively once replace half of the medium with fresh medium during the cultivation time
3.3 Stimulation (optional): Please do not add stimuli immediately after Nucleofection® as this may lead to increased cell mortality. For stimulation by antiCD3/antiCD28, transfer cells to an antibody coated plate (see chapter 1) containing fresh medium 5 hours post Nucleofection®, and incubate cells for another 43 hours. Alternatively you may add fresh medium containing IL-2 or other suitable stimuli 4 – 12 hours post Nucleofection®

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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