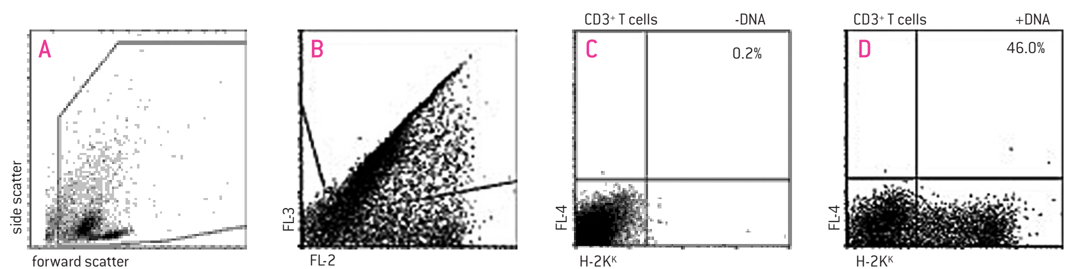
Amaxa® Human T Cell Nucleofector® Kit

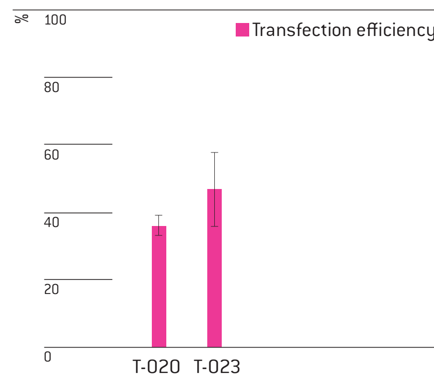
For stimulated human T cells

Stimulated CD3+ human T cells (small, round suspension cells (lymphocyte)) are a subpopulation of human peripheral blood mononuclear cells (PBMCs). PBMCs purified from fresh human blood samples treated with anticoagulant or from leucocyte rich buffy coat

Example for Nucleofection® of stimulated human T cells with H-2Kk cDNA



Separated CD3+ human T cells were stimulated for 5 days with anti-CD3/anti-CD28 antibodies. The cells were transfected by Nucleofection® using the Human T Cell Nucleofector® Kit and a plasmid encoding the mouse MHC class I heavy chain molecule H-2Kk. 24 hours post Nucleofection®, the cells were stained with a PE-coupled antibody directed against H-2Kk and analyzed by flow cytometry. CD3+ human T cells were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). H-2Kk expression is shown after Nucleofection® without (C) and with plasmid DNA (D).



Transfection efficiencies of human T cells stimulated with anti-CD3/anti-CD28 antibodies for 5 days. Cells were transfected by Nucleofection® with program T-023 or T-020 and 1 µg of a plasmid encoding the mouse MHC class I heavy chain molecule H-2Kk. 24 hours post Nucleofection® the cells were analyzed by flow cytometry.

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
- Anti-CD3/anti-CD28 coated 96-well and 6-well culture plates (see below) or coated culture plates 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 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 (1 – 5 x 10^6 cells per sample)

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 Nucleofection® of unstimulated T cells, please refer to the Optimized Protocol for Unstimulated Human T Cells.**

Coating of culture plates with anti-CD3 and anti-CD28 antibodies

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 mark, 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 may cause both a significant loss of stimulated T cells and reduced transfection efficiencies.**

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

Stimulation

1.12 Stimulate the isolated human T cells for 2 – 3 days prior Nucleofection® e.g. in 6-well plates coated with anti-CD3 antibody and anti-CD28 antibody (please see 1.1-1.2). Seed cells at 5 x 10^6 cells per ml

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 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 1.5 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 (**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 T Cell Nucleofector® Solution, as this reduces cell viability and gene transfer efficiency
2.6 Combine 100 µl of cell suspension with **1 – 2 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 **T-023** or **T-020** (T-20 or T-23 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% CO_2 incubator until analysis. Gene expression is often detectable after only 4 – 8 hours
3.2 Culture stimulated T cells post Nucleofection® in plates coated with anti-CD3 antibody and anti-CD28 antibody (see chapter 1)

Additional Information

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

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