Amaza® 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-2K⁺ 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-2K⁺. 24 hours post Nucleofection®, the cells were stained with a PE-coupled antibody directed against H-2K⁺ 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-2K⁺ expression is shown after Nucleofection® without (C) and with plasmid DNA (D).

Product Description

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>VPA-1002</th>
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<tbody>
<tr>
<td>Size (reactions)</td>
<td>25</td>
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<tr>
<td>Human T Cell Nucleofector® Solution</td>
<td>2.25 ml (2.05 ml + 10% overfill)</td>
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<tr>
<td>Supplement</td>
<td>0.5 ml (0.45 ml + 10% overfill)</td>
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<tr>
<td>pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)</td>
<td>30 µg</td>
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<tr>
<td>Certified cuvettes</td>
<td>25</td>
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<tr>
<td>Plastic pipettes</td>
<td>25</td>
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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.
**Optimized Protocol for Human T Cell**

**Required Material**

- 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 [SE8; Research Diagnostics Inc., Cat. No. 10R-CD28bHUµg/µl]; control antibody [purified mlgG(K); BD-Pharmingen, Cat. No. 554 721]; antibodies should be diluted in carbonate buffer (32 mM Na₂CO₃/16 mM NaHCO₃) 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 10⁶ 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. 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 mlgG(K)) at a final concentration 3 µg/ml] at 37°C/5% CO₂ for 5 hours
2. Wash the wells carefully three times with PBS/BSA
Optimized Protocol for Human T Cell

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

- $1 - 5 \times 10^6$ cells
- $1 - 5 \mu$g plasmid DNA (in $1 - 5 \mu$l H$_2$O or TE) or $2 \mu$g pmaxGFP® Vector or $30 - 300 \text{nM}$ siRNA
- $3 - 30 \text{pmol/sample}$
- $100 \mu$l 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 $1.5 \text{ml}$ of supplemented culture media and pre-incubate/equilibrate plates in a humidified $37^\circ\text{C}/5\%\text{ CO}_2$ incubator for at least 30 minutes

2.3 Count the cells and determine cell density

2.4 Centrifuge the required numbers of cells ($1 - 5 \times 10^6$ cells per sample) at $200\times g$ 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 $100 \mu$l 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 $100 \mu$l of cell suspension with $1 - 2 \mu$g 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 $\mu$l 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 $37^\circ\text{C}/5\%\text{ CO}_2$ 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](http://www.lonza.com/nucleofection-citations)

For more technical assistance, contact our Scientific Support Team:

<table>
<thead>
<tr>
<th>USA/Canada</th>
<th>Europe and Rest of World</th>
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<tbody>
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<td>Phone: 800 521 0390 (toll-free)</td>
<td>Phone: 49 221 99199 400</td>
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<td>Fax: 49 221 99199 499</td>
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<td>E-mail: <a href="mailto:scientific.support.eu@lonza.com">scientific.support.eu@lonza.com</a></td>
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50829 Cologne, Germany

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