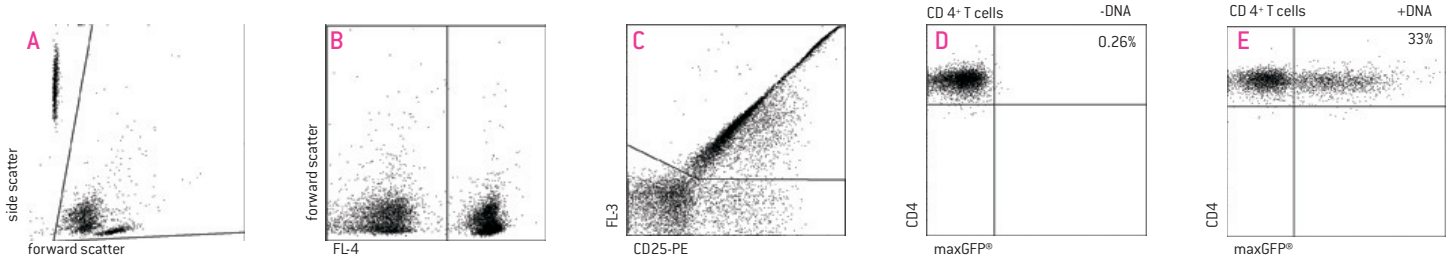
Amaxa® Mouse T Cell Nucleofector® Kit

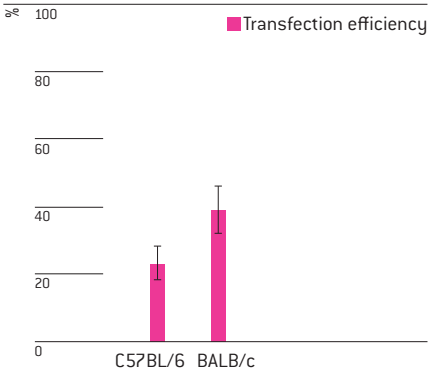
For T cells isolated from C57BL/6 & BALB/c mice

Evaluated for murine T cells isolated from C57BL/6 & BALB/c mice
This protocol is designed for murine lymphocytes or purified T cells (small round lymphoid cells) freshly isolated from spleens of BALB/c and C57BL/6 mice

Example for Nucleofection® of CD4+ mouse T cells (BALB/c) with maxGFP® Vector



Mouse T cells were freshly isolated from spleen, purified and nucleofected using the Mouse T Cell Nucleofector® Kit with a plasmid encoding maxGFP® Vector. 24 hours post Nucleofection®, the cells were stained with an antibody directed against CD4 and analyzed by flow cytometry. Lymphocytes were gated according to forward/side scatter (A). CD4 positive cells were stained with a CD4-APC and CD25-PE antibody. Dead cells were excluded by propidium iodide staining and gating (B, C). maxGFP® expression is shown after Nucleofection® without (D) and with plasmid DNA (E).



Average transfection efficiency of murine T cells (CD8+) 24 hours post Nucleofection®. Cells were transfected by Nucleofection® using program X-001 and 2.5 µg of pmaxGFP® Vector. Cell viability (% PI negative T cells) is usually around 35% (C57BL/6) to 55% (BALB/c) after 24 hours.

Product Description

Cat. No.	**VPA-1006**
Size (reactions)	25
Mouse 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
Mouse T Cell Nucleofector® Medium	100 ml
Medium Component A and B	1 ml each
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
- 12-well culture dish or culture system of choice
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- **Culture medium:** The Mouse T Cell 96-well Nucleofector® Kit is delivered with culture medium for mouse T cells. This medium is specially developed to provide consistent high-yield transfection results. The medium is used for the culture post Nucleofection® and is essential for survival of transfected mouse T cells. Using any other medium after Nuclofection® will most likely result in lower cell viability and transfection efficiency. Per 100 ml Mouse T Cell Nucleofector® Medium add 5 ml FCS, 1 ml 200 mM glutamine (2 mM final concentration) and 1 ml Medium Component A. This partially supplemented medium can be stored at 4°C for up to two weeks (alternatively it can be frozen in aliquots). Medium Component B must be added freshly for each experiment. Add 10 µl Medium Component B per ml partially supplemented Mouse T Cell Nucleofector® Medium to obtain the fully supplemented medium. Mouse T Cell Nucleofector® Medium can additionally be supplemented with 1000 U/ml penicillin and 1000 µg/ml Streptomycin [Lonza; Cat. No. 17-602E]
- **For isolation:** PBS containing 0.5% BSA (PBS/BSA)
- **For enrichment of T cells:** For enrichment or purification of T cell populations use only negative selection methods, otherwise you risk increasing cell mortality and activating the cells. We recommend the Pan T Cell Isolation Kit for mouse leucocytes [Miltenyi Biotec; Cat. No. 130-090-861]
- Prewarm appropriate volume of culture medium to 37°C (2 ml per sample)
- Appropriate number of cells ($5 \times 10^6 - 1 \times 10^7$ murine lymphocytes or $5 \times 10^5 - 1 \times 10^6$ purified untouched T cells per sample ; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection®

Notes

- **Transfection results may be donor-dependent**
- **C57BL/6 spleens are often smaller and provide fewer cells than BALB/c spleens, thus more spleens may be needed to provide necessary numbers of cells. Lymphocytes isolated from spleens of different animals of the same inbred strain and age can be pooled**
- **Prepare media, DNA, tubes etc. for Nucleofection® before preparing spleen cells**
- **Do not perform an erythrocyte lysis step as this will decrease cell viability**

Isolation of murine splenic lymphocytes

1.1 Excise spleens from 6 – 12 week old mice. One spleen yields up to 2 – 3 x 10^8 (BALB/c) or 0.8 – 1 x 10^8 (C57BL/6) cells. We recommend using freshly isolated organs (if necessary, whole spleens can be stored/transported in PBS/0.5% BSA)

1.2 Place one spleen into a 100 µm cell strainer atop a 50 ml Falcon™ tube. Use gentle suction of 5 or 10 ml pipette to manipulate spleen, as forceps are likely to rupture it
1.3 Use plunger from small syringe to crush spleen and force as much tissue as possible through strainer (process only 1 spleen/cell strainer)
1.4 Loosen cell strainer from top of Falcon™ tube to facilitate rinsing (this allows the solution to flow through the strainer more easily)
1.5 Rinse plunger and cell strainer with 10 ml PBS/0,5% BSA into tube with splenocytes
1.6 Pipette cell suspension onto 70 µm cell strainer atop a second 50 ml Falcon™ tube to remove clumps
1.7 Transfer the whole cell suspension (~10 ml) to a 15 ml Falcon™ tube. (The use of 15 ml Falcon™ tubes for centrifugation steps will lead to lower cell loss during removal of supernatant)
1.8 Centrifuge cell suspension at 90xg for 10 minutes (exceeding this speed will decrease cell viability)
1.9 Carefully remove supernatant, resuspend pellet in 10 ml PBS/BSA

Enrichment or purification of T cells

1.10 For enrichment or purification of T cell populations use only negative selection methods, otherwise you risk increasing cell mortality and activating the cells. We recommend the Pan T Cell Isolation Kit for mouse leucocytes]

2. Nucleofection®

One Nucleofection® Sample contains

5 x 10^6 – 1 x 10^7 murine lymphocytes or 5 x 10^5 – 1 x 10^6 purified untouched T cells
4 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2.5 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 – 30 pmol/sample)
100 µl Mouse 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 fully supplemented Mouse T Cell Nucleofector® Medium 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 x 10^6 – 1 x 10^7 murine lymphocytes or 5 x 10^5 – 1 x 10^6 purified T cells per sample) at 90xg 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 15 minutes in Mouse T Cell Nucleofector® Solution, as this reduces cell viability and gene transfer efficiency
2.6 Combine 100 µl of cell suspension with 4 µg DNA or 2.5 µg pmaxGFP® Vector 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 **X-001** (X-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 fully supplemented culture media to the cuvette and gently transfer the sample into the 12-well plate (final volume of 2 ml media per well). 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. If this is not the case, the incubation period may be prolonged to 24 – 48 hours

Note **Stimulation of T cells is possible within 3 – 24 hours post Nucleofection®. Stimulated T cells can be analyzed within 48 – 72 hours after transfection. We recommend using 5 µg/ml anti-CD3 and 2 µg/ml anti-CD28 antibodies for stimulation. It may be advisable to decrease the DNA amount to 0.5 – 1.5 µg per sample for stimulation experiments.**

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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