BioResearch

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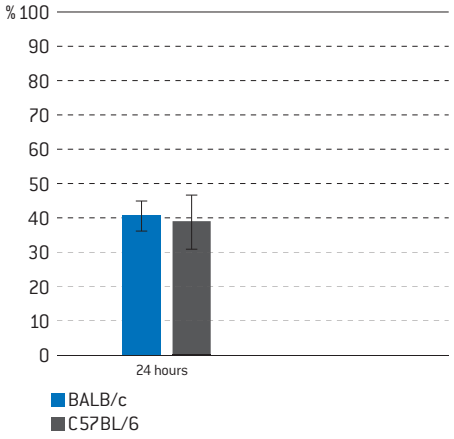
# Amaxa™ 4D-Nucleofector™ Protocol for Mouse T Cells

## For 4D-Nucleofector™ X Unit

Mouse T cells freshly isolated from spleens of BALB/c and C57BL/6 mice; small round lymphoid cells




### Example for Nucleofection™ of Mouse T Cells

Average transfection efficiency of mouse T cells 24 hours post Nucleofection™. Mouse T cells were transfected with program DN-100 and 0.5 µg of pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ [Becton Dickinson]. Cell viability (% PI negative cells) is usually around 30% (BALB/c) or 25% (C57BL/6) after 24 hours.

## Product Description

### Note

Optimal performance of this Nucleofector™ Kit requires the use of Mouse T Cell Nucleofector™ Medium (VZB-1001) for the post Nucleofection™ cell culture step!

### Recommended Kit(s)–P3 Primary Cell 4D-Nucleofector™ X Kit

| Cat. No. | V4XP-3012 | V4XP-3024 | V4XP-3032 |
|---|---|---|---|
| Transfection volume | 100 µl | 100 µl | 20 µl |
| Size [reaction] | 2 x 6 | 24 | 2 x 16 |
| Nucleofector™ Solution | 2 x 0.675 ml (0.492 ml + 27% overfill) | 2.25 ml (1.968 ml + 13% overfill) | 0.675 ml (0.525 ml + 22% overfill) |
| Supplement | 2 x 0.15 ml (0.108 ml + 27% overfill) | 0.5 ml (0.432 ml + 13% overfill) | 0.15 ml (0.115 ml + 22% overfill) |
| pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0) | 50 µg | 50 µg | 50 µg |
| Single Nucleocuvette™ (100 µl) | 12 | 24 | - |
| 16-well Nucleocuvette™ Strips (20 µl) | - | - | 2 |

### 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.

### Note

4D-Nucleofector™ Solutions can only be used with Nucleovettes™ (conductive polymer cuvettes), i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ Device. They are not compatible with the Nucleofector™ II/2b Device.

# 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

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 100 µl single Nucleocuvette™ or 20 µl 16-well Nucleocuvette™ Strips
- **Compatible tips for 20 µl Nucleocuvette™ Strips**: epT.I.P.S. [US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266], Matrix TallTips® [Matrix Technologies Corp., Cat. No. 7281] or LTS Tips [Rainin Instruments, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S]. Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- **Supplied pmaxGFP™ Vector, stock solution 1 µg/µl**

### Note

For positive control using pmaxGFP™, dilute the stock solution to an appropriate working concentration. Further details are provided in table 3 of this Optimized Protocol. The volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µl for 20 µl reactions; 10 µl for 100 µl reactions).

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260:A280 ratio should be at least 1.8
- Cell culture plates of your choice
- **PBS/BSA for isolation:** PBS containing 0.5% 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]
- **Culture medium:** For optimal performance of this Nucleofector™ Kit it is highly recommended to use Mouse T Cell Nucleofector™ Medium (VZB-1001) for cell culture steps post Nucleofection™. This medium is specially developed to provide consistent high-yield transfection results 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. To complete the medium add 5 ml FCS, 1 ml 200 mM glutamine (2 mM final concentration) and 1 ml Medium Component A per 100ml medium. 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. Therefore 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

# 1. Pre Nucleofection™

## Preparation of Cells and Cell Culture

### Notes

1. 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.
2. Prepare media, DNA, tubes and further required material for Nucleofection™ before preparing spleen cells.

## Isolation of Murine Splenic Lymphocytes

1.1 Excise spleens from 6–12 week old mice. One spleen yields up to $2–3 \times 10^8$ (BALB/c) or $0.8–1 \times 10^8$ (C57BL/6) splenic lymphocytes. 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

### Note

Do not perform an erythrocyte lysis step as this will decrease cell viability.

## 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™

For Nucleofection™ Sample contents and recommended Nucleofector™ Program, please refer to Table 3.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)
2.3 Select/Check for the appropriate Nucleofector™ Program (see table 3)
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media (see table 4) and pre-incubate/equilibrate plates in a humidified 37°C/5% $CO_2$ incubator
2.5 Pre-warm an aliquot of culture medium to 37°C (see table 4)
2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)
2.7 Count an aliquot of the cells and determine cell density
2.8 Centrifuge the required number of cells (see table 3) at 90xg for 10 minutes at room temperature. Remove supernatant completely
2.9 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see table 3)
2.10 Prepare mastermixes by dividing cell suspension according to number of substrates
2.11 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
2.12 Transfer mastermixes into the Nucleocuvette™ Vessels

### Note

As leaving cells in Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability it is important to work as quickly as possible. Avoid air bubbles while pipetting.

2.13 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
2.14 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.15 Start Nucleofection™ Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.16 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.17 Resuspend cells with pre-warmed fully supplemented Mouse T Cell Nucleofector™ Medium (for recommended volumes see table 5). Mix cells by gently pipetting up and down two to three times. When working with the 100 µl Nucleocuvette™ use the supplied pipettes and avoid repeated aspiration of the sample
2.18 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

## 3. Post Nucleofection™

3.1 Incubate the cells in humidified 37°C/5% $CO_2$ incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 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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### References

1. Shi GX et al. [2002] J Immunol 169(5): 2507-15
2. Tolnay M et al. [2002] J Immunol 169(11): 6236-43

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## Table 1: Volumes required for a single reaction

| | 100 µl Single Nucleocuvette™ | 20 µl Nucleocuvette™ Strip |
|---|---|---|
| **Volume of Nucleofector™ Solution** | 82 µl | 16.4 µl |
| **Volume of Supplement** | 18 µl | 3.6 µl |

## Table 2: Required amounts of cells and media for Nucleofection™

| | 100 µl Single Nucleocuvette™ | 20 µl Nucleocuvette™ Strip |
|---|---|---|
| **Culture medium per sample post Nucleofection™ (for transfer and culture)** | 2 ml | 230 µl |
| **Cell number per Nucleofection™ Sample** | 5 x $10^6$ -1 x $10^7$ murine lymphocytes or<br>5 x $10^5$–1 x $10^6$ purified untouched T cells<br>(Lower or higher cell numbers may influence transfection results) | 2 x $10^6$ cells<br>(Cell numbers less than 5 x $10^4$ may lead to a major decrease in transfection efficiency and viability) |

## Table 3: Contents of one Nucleofection™ Sample and recommended program

| | | 100 µl Single Nucleocuvette™ | 20 µl Nucleocuvette™ Strip |
|---|---|---|---|
| **Cells** | | 5 x $10^6$–1 x $10^7$ murine lymphocytes or<br>5 x $10^5$–1 x $10^6$ purified untouched T cells | 2 x $10^6$ |
| **Substrate*** | **pmaxGFP™ Vector** | 2.5 µg | 0.5 µg |
| **or** | **plasmid DNA (in $H_2O$ or TE)** | 4 µg | 0.2–1 µg |
| **or** | **siRNA** | 30–300nM siRNA<br>(3–30 pmol/sample) | 30–300nM siRNA<br>(0.6–6 pmol/sample) |
| **P3 Primary Cell 4D-Nucleofector™ X Solution** | | 100 µl | 20 µl |
| **Program** | | DN-100 | DN-100 |

* Volume of substrate should comprise maximum 10% of total reaction volume

## Table 4: Culture volumes required for post Nucleofection™ Steps

| | 100 µl Single Nucleocuvette™ | 20 µl Nucleocuvette™ Strip* |
|---|---|---|
| **12-well culture plate** | 1.5 ml | - |
| **96-well culture plate** | - | 150 µl |
| **Culture medium to be added to the sample post Nucleofection™** | 500 µl | 80 µl |

* Maximum cuvette volume 200 µl

## Table 5: Recommended volumes for sample transfer into culture plate

| | 100 µl Single Nucleocuvette™ | 20 µl Nucleocuvette™ Strip* |
|---|---|---|
| **Culture medium to be added to the sample post Nucleofection™** | 500 µl | 80 µl |
| **Volume of sample transferred to culture plate** | complete sample (use supplied pipettes) | 50 µl |

* Maximum cuvette volume 200 µl