BioResearch

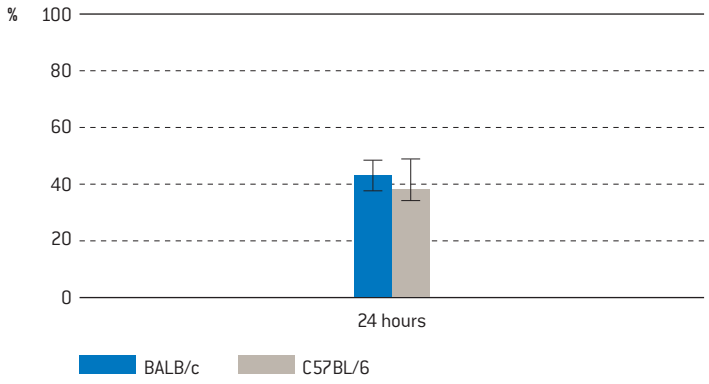
Lonza

Amaxa™ 96-well Shuttle™ Protocol for Mouse T Cells

Cell Description

This protocol is designed for freshly isolated T cells from spleens of BALB/c and C57BL/6 mice; small round lymphoid cells.

Example for 96-well Nucleofection™ of Mouse T Cells



Average transfection efficiency of mouse T cells 24 hours post Nucleofection™. Mouse T cells were transfected with program 96-DN-100 and 0.5 µg of pmaxGFP™ Vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability (% PI negative cells) is usually around 30 % (BALB/c) or 25 % (C57BL/6) after 24 hours.

Product Description

Recommended Kits

P3 Primary Cell 96-well Nucleofector™ Kits

Cat. No.	V4SP-3096
Size (reactions)	1x96
P3 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	1

Cat. No.	V4SP-3960
Size (reactions)	10x96
P3 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate(s)	10

Note

Optimal performance of this 96-well Nucleofector™ Kit requires the use of Mouse T Cell Nucleofector™ Medium (VZB-1001) for the post Nucleofection™ cell culture step!

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

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. 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.

- Nucleofector™ 96-well Shuttle™ System (4D-Nucleofector™ System; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plate(s)
- Nucleocuvette™ compatible tips: 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 Instrument, 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
Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- 96-well culture plates or 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 96-well 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 [Lonza; Cat. No. 17-602E]
- Prewarm appropriate volume of culture medium to 37°C (230 µl per sample)
- Appropriate number of cells ($2x10^6$ cells per sample; cell numbers less than $5x10^4$ may lead to a major decrease in transfection efficiency and viability)

1. Pre Nucleofection™

Preparation of Cells and Cell Culture

Notes
- 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 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 $2–3x10^8$ (BALB/c) or $0.8–1x10^8$ (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™

One Nucleofection™ Sample Contains

- $2x10^6$ cells
- 0.2–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.5 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P3 Primary Cell 96-well Nucleofector™ Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution

2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see device and software manuals)

2.3 Select the appropriate 96-well Nucleofector™ Program **96-DN-100**

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of culture medium, e.g. 150 µl* (see note at the end of this chapter) for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

2.5 Pre-warm an aliquot of fully culture medium to 37°C (80 µl per 2.5 sample*)

2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Count an aliquot of the enriched mouse T cell population and determine cell density

2.8 Centrifuge the required number of cells ($2x10^6$ cells per sample) at 90xg for 10 minutes at room temperature. Remove supernatant completely

2.9 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B: Multiple substrates (e.g. library transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well 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.

2.10 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well

2.11 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle. Well "A1" must be in upper left position

2.12 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)

2.13 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer

2.14 Resuspend cells with 80 µl* (recommendation for 96-well plates) or desired volume of pre-warmed medium (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times.

2.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 50 µl of resuspended cells to 150 µl pre-warmed medium prepared in 96-well culture plates*

***Note**

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ Results in most cases. However, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

3. Post Nucleofection™

3.1 Incubate the cells in a 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

Up-To-Date List of all Nucleofector™ References

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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