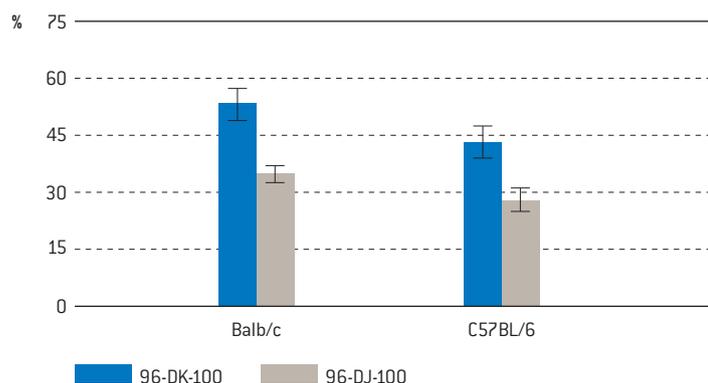


Amaxa™ 96-well Shuttle™ Protocol for Immature Mouse Dendritic Cells

Cell Description

Progenitor cells derived from bone marrow; non-adherent or loosely adherent cells of irregular shape with typical protrusions (“dendrites”) of variable shape and length.

Example for Nucleofection™ of immature mouse dendritic cells



Average transfection efficiency of immature mouse dendritic cells 24 hours post Nucleofection™. 5×10^4 immature mouse dendritic cells were transfected with program 96-DK-100 (high efficiency) or 96-DJ-100 (high viability) and 0.4 μg of pmaxGFP™ Vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability was approximately 74% for 96-DK-100 and 98% for 96-DJ-100 after 24 hours [% PI-negative compared to untreated].

Product Description

Recommended Kits

P4 Primary Cell 96-well Nucleofector™ Kits

Cat. No.	V4SP-4096
Size (reactions)	1×96
P4 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 $\mu\text{g}/\mu\text{l}$ in 10 mM Tris pH 8.0)	50 μg
Nucleocuvette™ Plate (s)	1

Cat. No.	V4SP-4960
Size (reactions)	10×96
P4 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1.0 $\mu\text{g}/\mu\text{l}$ in 10 mM Tris pH 8.0)	50 μg
Nucleocuvette™ Plate (s)	10

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.

Notes

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 (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plate(s)
- 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

Note

As contamination of the DNA with low molecular weight compounds may reduce cell viability, we strongly recommend performing an additional purification step after using a plasmid purification kit. Therefore precipitate the purified DNA twice using 20 % PEG/2.5 M NaCl (final concentration).

- 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
- 96-well culture plates or culture plates of your choice
- Culture medium I: RPMI 1640 (Lonza; Cat. No. 12-167F) supplemented with 10 % calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml Penicillin, 2 mM glutamine and 2000 U/ml GM-CSF (BD Pharmingen; Cat. No.: 554586)
- Culture medium II: Culture medium I without GM-CSF
- Prewarm appropriate volume of culture medium to 37°C (190 µl per sample)
- Appropriate number of cells (5×10⁴ cells per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Preparation of Cells and Cell Culture

Preparation of Bones

- 1.1 Carefully remove the femurs and tibia of freshly prepared mouse hindlegs using forceps and scissors. Cut off both ends of the bones

Isolation of Dendritic Cells

- 1.2 Use a 27G needle mounted to a 5 ml syringe to gently flush the bone marrow into a petri dish. Use 2–3 ml culture medium II per bone
- 1.3 Count the viable cells
- 1.4 Spin down cells at 300×g for 10 minutes at RT and discard the supernatant
- 1.5 Resuspend the cell pellet in culture medium I to reach a cell density of 1×10⁶ cells/ml
- 1.6 Transfer the cells into 24-well plates (1 ml/well) and incubate them in a 37°C incubator with a 5 % CO₂ atmosphere

Note

To yield a high number of functional dendritic cells it is necessary to maintain a sufficient level of GM-CSF. Fresh culture medium I (containing GM-CSF) should be added every second day.

- 1.7 On day 2, carefully remove 700 µl of the cell medium from each well and replace it by fresh culture medium I, to maintain an appropriate GM-CSF concentration
- 1.8 Remove and discard the cell medium completely on day 3. Wash the cells carefully with 500 µl per well using culture medium II to remove residual non adherent cells and add 1 ml fresh culture medium I per well
- 1.9 Incubate the cells at 37°C in an incubator with 5 % CO₂ atmosphere
- 1.10 Harvest the dendritic cells on day 6 by collecting non adherent cells and loosely adherent cells. To release loosely adherent cells, wash off the cells thoroughly by pipetting with culture medium I. Discard the adherent cells

2. Nucleofection™

One Nucleofection™ Sample Contains

- 5×10^4 cells
- 0.4–0.8 µg plasmid DNA (in 1–2 µl H₂O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P4 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-DK-100** (for high efficiency) or **96-DJ-100** (for high viability)
- 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of culture medium I, e.g. 110 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% CO₂ incubator
- 2.5 Pre-warm an aliquot of culture medium I to 37°C (80 µl per sample*)
- 2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector or 30 nM–300 nM siRNA (0.6–6 pmol/sample)
- 2.7 Count an aliquot of the cells and determine cell density
- 2.8 Centrifuge the required number of cells (5×10^4 cells per sample) at $300 \times g$ 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 culture medium I (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 90 µl of resuspended cells to 110 µl pre-warmed culture medium I 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% CO₂ 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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Reference

1. Inaba K. et al, [1992] J. Exp. Med. 176: 1693-1702

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