BioResearch

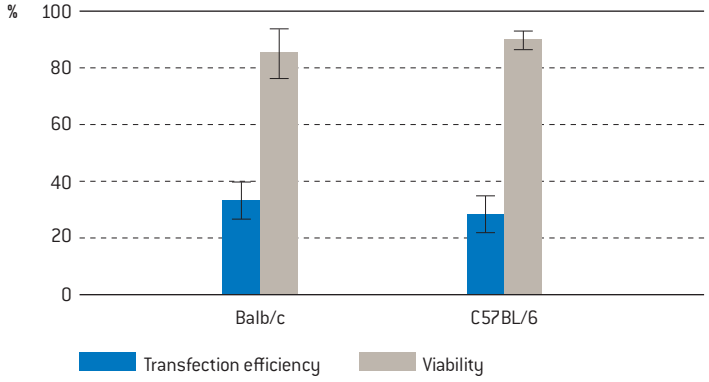
Lonza

Amaxa™ HT Nucleofector™ protocol for mature mouse dendritic cells

Cell description

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

Example for Nucleofection™ of mature mouse dendritic cells



Average transfection efficiency and viability of mature mouse dendritic cells 24 hours post Nucleofection™. 2.5×10^4 mature mouse dendritic cells were transfected with program DN-107-AA 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 85 % after 24 hours (compared to PI negative cells of untreated control).

Product description

Recommended kits

P3 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-3002
Size (reactions)	2×384
P3 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	150 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-3010
Size (reactions)	10×384
P3 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	150 µg
384-well 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.

Note

HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™ system, the 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.

- HT Nucleofector™ system
- Supplemented HT Nucleofector™ solution at room temperature
- Supplied 384-well 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).

- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use compatible tips such as: 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). Please ensure the chosen pipette tips reach the bottom of the 384-well Nucleocuvette™ wells without getting stuck
- 384-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), 100µg/ml streptomycin, 100U/ml Penicilin, 2 mM glutamine and 2000 U/ml GM-CSF (BD Pharmingen; Cat. No.: 554586)
- Culture medium II: Culture medium I without GM-CSF
- Maturation medium: Culture medium I with 0.1 µg/ml LPS (Sigma, Cat. No. L-9764)
- Pre-warm appropriate volume of culture medium to 37°C (186 µl per sample)
- Appropriate number of cells (2.5×10^4 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 hind legs 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 1×10^6 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 % CO_2 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 500µl 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 % CO_2 atmosphere

Maturation of dendritic cells

1.10 To generate mature dendritic cells stimulate the immature cells with 0.1 µg/ml LPS. Therefore remove 1 ml of the cell supernatant on day 6 and replace it by 1 ml plating medium supplemented with 0.1 µg LPS/ml
1.11 Incubate the cells for one more day
1.12 Harvest the dendritic cells on day 7 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

1. Nucleofection™

One Nucleofection™ sample contains

- 2.5×10^4 cells
- 0.4–0.8 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P3 primary cell HT Nucleofector™ solution

1.1 Please make sure that the entire supplement is added to the Nucleofector™ solution

1.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details please refer to the HT Nucleofector™ manuals)

1.3 Select the appropriate thel HT Nucleofector™ program DN-107-AA

1.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of culture medium I, e.g. 146 µl* (see note at the end of this chapter) for one well of a 384-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

1.5 Pre-warm an aliquot of culture medium I to 37°C (40 µl per sample)

1.6 Prepare 0.4–0.8 µg plasmid DNA or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)

1.7 Count an aliquot of the cells and determine cell density

1.8 Centrifuge the required number of cells (2.5×10^4 cells per sample) at 300×g for 10 minutes at room temperature. Remove supernatant completely

1.9 Resuspend the cell pellet carefully in 20 µl room temperature HT 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 384-well Nucleocuvette™ plates

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

- Pipette 20 µl of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. As leaving cells in 384-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.

1.10 Briefly shake 384-well Nucleocuvette™ plates with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the well without air bubbles. Alternatively, thoroughly tap the 384-well Nucleocuvette plate.

1.11 Place 384-well Nucleocuvette™ plate with closed lid on to the carousel of the plate handler. Well "A1" must be in upper left position

1.12 Start the HT Nucleofection™ process by clicking "Start" in the HT Nucleofector™ software (for details please refer to the HT Nucleofector™ manuals)

1.13 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel

1.14 Resuspend cells with 40 µl* (recommendation for 96-well plates) or desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times

1.15 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 54 µl of resuspended cells to 146 µl pre-warmed culture medium prepared in 384-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 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.

2. Post Nucleofection™

2.1 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

Technical assistance and scientific support

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Reference

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

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