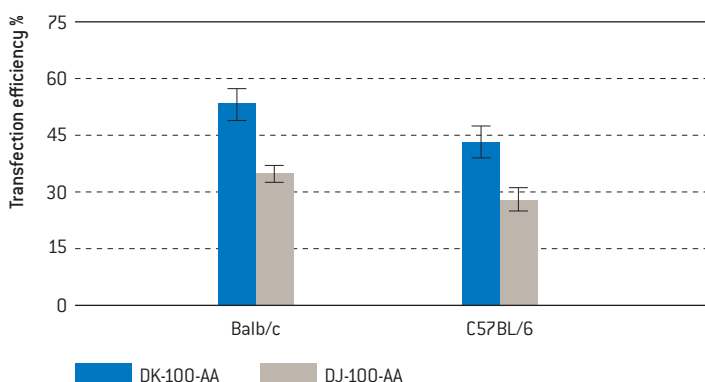


# Amaxa™ HT Nucleofector™ protocol for immature mouse dendritic cells

## 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™.**  $5 \times 10^4$  immature mouse dendritic cells were transfected with program DK-100-AA (high efficiency) or DJ-100-AA (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 DK-100-AA and 98 % for DJ-100-AA after 24 hours [% PI-negative compared to untreated].

## Product description

### Recommended kits

P4 primary cell HT Nucleofector™ kits

|  |           |
|--|-----------|
| Cat. No.   | V5SP-4002 |
| Size (reactions)                                 | 2×384     |
| P4 primary cell HT Nucleofector™ solution        | 22.5 ml   |
| Supplement                                       | 5 ml      |
| pmaxGFP™ vector (1.0 µg/µl in 10 mM Tris pH 8.0) | 50 µg     |
| 384-well Nucleocuvette™ plate(s)                 | 2         |

|  |           |
|--|-----------|
| Cat. No.   | V5SP-4010 |
| Size (reactions)                                 | 10×384    |
| P4 primary cell HT Nucleofector™ solution        | 90 ml     |
| Supplement                                       | 20 ml     |
| pmaxGFP™ vector (1.0 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 expiry 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

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

- 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.

### 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).

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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 Penicilin, 2 mM glutamine and 2000 U/ml GM-CSF (BD Pharmingen; Cat. No.: 554586)
- Culture medium II: Culture medium I without GM-CSF
- Pre-warm appropriate volume of culture medium to 37°C (190 µl per sample)
- Appropriate number of cells (5×10<sup>4</sup> 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 1×10<sup>6</sup> 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<sub>2</sub> 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 % CO<sub>2</sub> 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

## 1. Nucleofection™

### One Nucleofection™ sample contains

- $5 \times 10^4$  cells
- 0.4–0.8 µg plasmid DNA (in 1–2 µl H<sub>2</sub>O or TE) or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P4 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 HT Nucleofector™ program **DK-100-AA** (for high efficiency) or **DJ-100-AA** (for high viability)
- 1.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of culture medium I, e.g. 186 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<sub>2</sub> incubator
- 1.5 Pre-warm an aliquot of culture medium I to 37°C (40 µl per sample)
- 1.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ vector or 30 nM–300 nM siRNA (0.6–6 pmol/sample)
- 1.7 Count an aliquot of the cells and determine cell density
- 1.8 Centrifuge the required number of cells ( $5 \times 10^4$  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. Use a liquid handling system or at least a multi-channel pipette with suitable pipette tips. As leaving cells in HT 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 the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate
- 1.11 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1" must be in upper left position
- 1.12 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details 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 desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40 µl of pre-warmed media\*
- 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 media

### \* 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<sub>2</sub> 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](http://www.lonza.com/nucleofection-citations)

### Technical assistance and scientific support

#### USA/Canada

Tel 800 521 0390 (toll-free)

Fax 301 845 8338

[scientific.support@lonza.com](mailto:scientific.support@lonza.com)

#### Europe and Rest of World

Tel + 49 221 99199 400

Fax + 49 221 99199 499

[scientific.support.eu@lonza.com](mailto:scientific.support.eu@lonza.com)

## Reference

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

### [www.lonza.com](http://www.lonza.com)

---

Lonza Cologne GmbH – 50829 Cologne, Germany

Please note that the Amaxa™ Nucleofector™ technology is not intended to be used for diagnostic purposes or for testing or treatment in humans. The Nucleofector™ technology, comprising Nucleofection™ process, Nucleofector™ device, Nucleofector™ solutions, Nucleofector™ 96-well Shuttle™ system, 96-well Nucleocuvette™ plates and modules, HT Nucleofector™ and 384-well Nucleocuvette™ plates is covered by patent and/or patent-pending rights owned by Lonza Cologne GmbH. Amaxa, Nucleofector, Nucleofection, 96-well Shuttle, Nucleocuvette and maxGFP are either registered trademarks or trademarks of the Lonza Cologne GmbH in Germany and/or U.S. and/or other countries. TallTips are a registered trademark of Matrix Technologies Corporation. Other product and company names mentioned herein are the trademarks of their respective owners.

This kit contains a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as a positive control with this Lonza product only. Any use of the proprietary nucleic acid or protein other than as a positive control with this Lonza product is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at [license@evrogen.com](mailto:license@evrogen.com). The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242. The use of this product in conjunction with materials or methods of third parties may require a license by a third party. User shall be fully responsible for determining whether and from which third party it requires such license and for the obtainment of such license. No statement is intended or should be construed as a recommendation to infringe any existing patent.