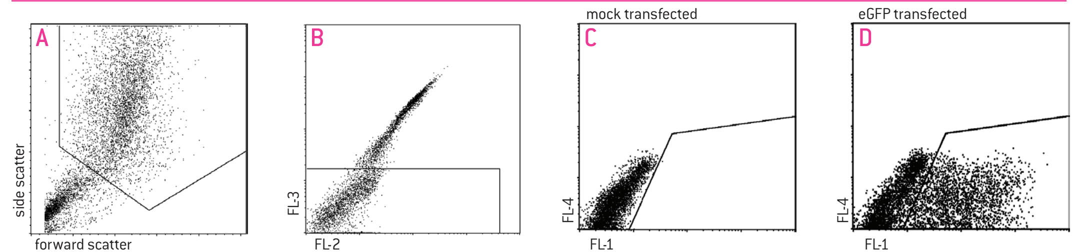
Lonza

# Amaxa® Human Dendritic Cell Nucleofector® Kit

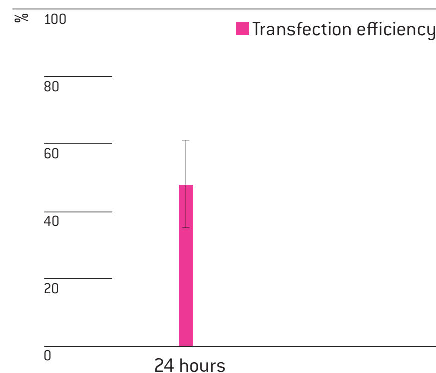
## For human dendritic cells

Immature or mature dendritic cells generated by in vitro differentiation of $CD14^+$ monocytes (subpopulation of peripheral blood mononuclear cells, PBMC) using IL-4 and GM-CSF containing medium. Dendritic cells are loosely adherent or non-adherent cells of irregular shape with typical protrusions ("dendrites") of variable shape and length.

**Example for Nucleofection® of human dendritic cells with eGFP cDNA.**




**Human dendritic cells were differentiated from monocytes using 100 ng/ml IL-4 and 50 ng/ml GM-CSF.** On day 6 cells were transfected by Nucleofection® with 5 µg plasmid DNA encoding the green fluorescent protein (eGFP) using the Human Dendritic Cell Nucleofector® Kit. 24 hours post Nucleofection® cells were analyzed by flow cytometry (FACS). Dendritic cells were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). eGFP expression of dendritic cells is shown after Nucleofection® with a control plasmid (C) and with a plasmid encoding eGFP (D) (courtesy of Dr. Esen and Prof. Radbruch, Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, Germany).




**Transfection efficiencies of immature dendritic cells 24 hours post Nucleofection®.** Cells were transfected by Nucleofection® with program U-002 and 5 µg of a plasmid encoding eGPF.

## Product Description

| Cat. No. | **VPA-1004** |
|---|---|
| Size (reactions) | 25 |
| Human Dendritic Cell Nucleofector® Solution | 2.25 ml (2.05 ml + 10% overfill) |
| Supplement | 0.5 ml (0.45 ml + 10% overfill) |
| pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0) | 30 µg |
| Certified cuvettes | 25 |
| Plastic pipettes | 25 |
| 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. |

## 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. For a single reaction use 82 µl of Nucleofector® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.**

- Nucleofector® Device
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- 12-well culture dish or culture system of your choice
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- **Culture medium I:** RPMI 1640 [Lonza; Cat. No. 12-167F] supplemented with 10% fetal calf serum (FCS), 100 µg/ml streptomycin, 100 U/ml penicillin, 2 mM UltraGlutamine I [Lonza, Cat. no. BE17-605E/U1] and 1 mM sodium pyruvate
- **Culture medium II:** Culture medium I supplemented with 50 ng/ml GM-SCF [Promocell; Cat. No. C-60420] and 100 – 200 ng/ml IL-4 [Promocell; Cat. No. C-61410]
- **Culture medium III:** Culture medium II plus 10-50 ng/ml TNF-alpha
- **For isolation:** PBS containing 0.5% BSA (PBS/BSA); Ficoll-Paque™ Plus [GE Healthcare; Cat. No. 17-1440-03]
- Prewarm appropriate volume of culture medium I to 37°C (500 µl per sample)
- Appropriate number of cells (0.5 – 2 x $10^6$ cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection®

Notes

- **Transfection results may be donor-dependent.**
- **Please make sure that all components you use for dendritic cell culture and transfection e.g. PBS, FCS and especially DNA are LPS-free.**
- **Please follow the outlines given in this protocol carefully to ensure reproducibility.**
- **Isolation or culture methods different from those mentioned below (e.g. cold aggregation) may result in lower transfection efficiency and viability.**
- **Alternatively to plastic adherence, monocytes can also be isolated by magnetic separation.**
- **For transfection of CD34-derived dendritic cells, similar results as for monocyte derived dendritic cells have been reported using the Human Dendritic Cell Nucleofector® Kit and Program U-002.**

### Blood samples

1.1 The peripheral blood or buffy coat should be supplemented with anticoagulants and should not be older than 8 hours. For isolation of monocytes, e.g. by plastic adherence (see protocol below), freshly isolated PBMCs should be used. The samples should be diluted with 2 – 4 volumes of PBS/BSA

### Preparation of PBMC

1.2 Pipet 15 ml Ficoll-Paque™ Plus in a 50 ml conical tube
1.3 Overlay Ficoll- Paque™ Plus with 35 ml blood sample and centrifuge at 750xg for 20 minutes at 20°C in a swinging-bucket rotor without brake
1.4 Remove the upper layer leaving the mononuclear cell layer undisturbed at the interphase. Carefully transfer the interphase cells (lymphocytes and monocytes) to a new 50 ml conical tube
1.5 Add PBS/BSA to 50 ml mark, mix and centrifuge at 350xg for 10 minutes at 4°C. Remove the supernatant carefully
1.6 Resuspend the cell pellet in 25 ml of PBS/BSA and centrifuge at 160xg for 15 minutes at 4°C. Remove the supernatant carefully
1.7 Resuspend the cell pellet in 25 ml PBS/BSA and centrifuge at 300xg for 10 minutes at 4°C. Remove the supernatant carefully
1.8 Resuspend cell pellet in 5 ml PBS/BSA and count the cells

### Generation of immature monocyte-derived dendritic cells (adapted from Sallusto and Lanzavecchia)

1.9 Prepare fresh PBMC as described above
1.10 Plate 1 – 1.5 x 10 PBMC in a T162 flask with 25 ml supplemented culture medium I
1.11 Incubate for 2 – 3 hours in a humidified 37°C/5% $CO_2$ incubator
1.12 Discard medium containing non-adherent cells
1.13 Wash the adherent cells (mainly $CD14^+$ monocytes) three times with 20 ml PBS (Optional: culture monocytes in culture medium I overnight at 37°C. Thereafter, monocytes either float or can be detached easily, whereas contaminating cells (e.g. fibroblasts) remain adherent. Harvest monocytes by short centrifugation and continue as follows)
1.14 Add 25 ml culture medium II. Cell density should be between 1 – 3 x $10^5$/ml
1.15 Culture cells in a humidified 37°C/5% $CO_2$ incubator for 6 days. We recommend replacing medium once after 3 days with fresh supplemented culture medium II. Save all non-adherent or loosely adherent cells by centrifuging the removed culture medium 10 min at 200xg and adding the pellet to the fresh culture medium
1.16 After 6 days, the loosely adherent or non-adherent cells (approximately 3 – 6 x $10^6$ per T162) should display typical dendritic cell morphology and surface markers (CD1a, CD80, CD86, HLA-DR). If possible, check an aliquot of cells for expression of these markers

### Generation of mature monocyte-derived dendritic cells

Mature dendritic cells can be generated from immature monocyte derived dendritic cells by adding 10 – 50 ng/ml TNF-$\alpha$ to the culture (culture medium III) and incubating for 1 or 2 more days. Mature dendritic cells should display CD83 as an additional surface marker and upregulation of HLA-DR, CD80 and CD86

## 2. Nucleofection®

| One Nucleofection® Sample contains |
| --- |
| 0.5 – 2 x $10^6$ cells |
| 1 – 5 µg plasmid DNA (in 1 – 5 µl $H_2O$ or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA (3 – 30 pmol/sample) |
| 100 µl Human Dendritic Cell Nucleofector® Solution |

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

2.2 Prepare 12-well plates by filling appropriate number of wells with 0.5 ml of supplemented culture medium II and pre-incubate/equilibrate plates in a humidified 37°C/5% $CO_2$ incubator

2.3 Count the cells and determine cell density

2.4 Centrifuge the required numbers of cells (**0.5 – 2 x $10^6$ cells per sample**) at **200xg for 10 minutes** at room temperature. Discard supernatant completely so that no residual PBS/BSA covers the cell pellet

2.5 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample. Avoid storing the cell suspension longer than 15 min in Human Dendritic Cell Nucleofector® Solution, as this reduces cell viability and gene transfer efficiency

2.6 Combine 100 µ l of cell suspension with **1 – 5 µ g DNA** or appropriate amount of siRNA or other substrates

2.7 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap

2.8 Select the appropriate Nucleofector® Program **U-002** (U-02 for Nucleofector® I Device)

2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program

2.10 Take the cuvette out of the holder once the program is finished

2.11 Add ~500 µ l of the pre-equilibrated supplemented culture medium I to the cuvette and **gently** transfer the sample into the 12-well plate (final volume of 1 ml media per well/sample; mix of culture media I and II). Use the supplied pipettes and avoid repeated aspiration of the sample

## 3. Post Nucleofection®

3.1 Incubate the cells in humidified 37°C/5% $CO_2$ incubator until analysis. Gene expression is often detectable after only 4 – 8 hours

Note **After 48 hours cell viability may decrease significantly.**

## Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
**www.lonza.com/nucleofection-citations**

### For more technical assistance, contact our Scientific Support Team:

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### References

1. Sallusto, F and Lanzavecchia, A (1994). J Exp Med 179:1109-1118

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Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

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