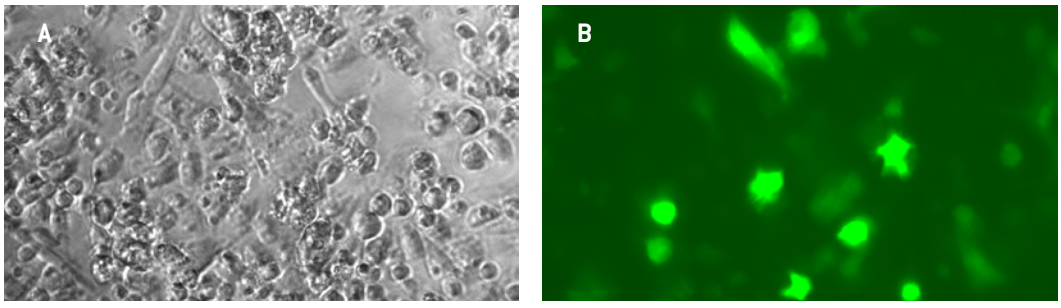
# Amaxa® Mouse Dendritic Cell Nucleofector® Kit

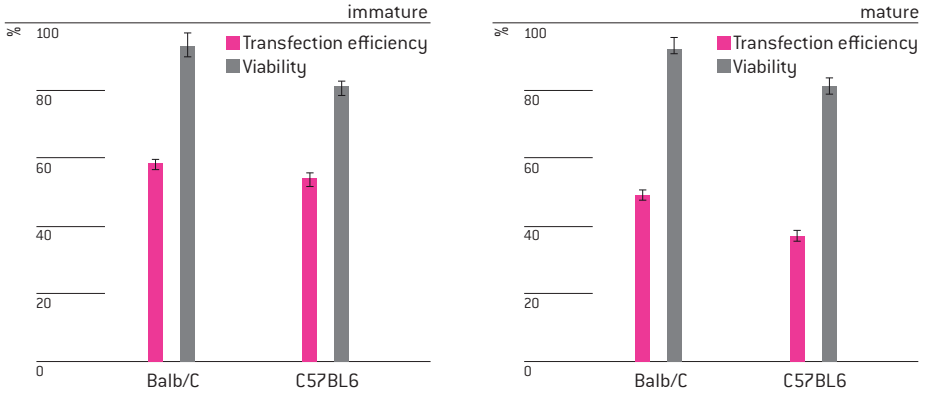
## For Immature and Mature Mouse Dendritic Cells

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

### Example for Nucleofection® of immature and mature mouse dendritic cells




**Example showing typical Nucleofection® results of mouse D dendritic cells.** Immature mouse dendritic cells were transfected with 2 µg pmaxGFP® Vector using the Mouse Dendritic Cell Nucleofector® Kit. 24 hours post Nucleofection® cells were analyzed by light (A) or fluorescence microscopy (B).




**Transfection efficiency and cell viability of mouse dendritic cells 24 hours post Nucleofection®.** 2.5 x $10^5$ immature cells were transfected with Nucleofector® Program Y-001 or 2.5 x $10^5$ mature cells were transfected with program AN-001 using 2 µg pmaxGFP® Vector. Cells were analyzed 24 hours post Nucleofection® by flow cytometry. Cell viability was determined as % negative cells compared to untreated control.

## Product Description

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| Cat. No. | **VPA-1011** |
| Size (Reactions) | 25 |
| Mouse DC 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® II Device; Software requirements: version S3-7 or higher for Nucleofector® II Device; version S4-4 or higher for Nucleofector® II Device, serial version "s"
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- 48-well culture dish or culture system of your choice
- **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 glutamine and 2000U/ml GM-CSF [BD Pharmingen; Cat. No. 554586]
- **Culture medium II:** Culture medium I without GM-CSF
- **Maturation medium**: Culture medium with 0.1 µg/ml LPS [Sigma; Cat. No. L-9764]
- Prewarm appropriate volume of culture media to 37°C (0.8 ml per sample)
- Appropriate number of cells (2.5 x $10^5$ cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection®

**Note** **Transfection results may be donor – dependent.**

### 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 immature 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. Count the viable cells

1.3 Spin down cells at 300xg for 10 minutes at RT and discard the supernatant

1.4 Resuspend the cell pellet in culture medium I to reach a cell density of 1 x $10^6$ cells/ml

1.5 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 medium containing GM-CSF should be added every second day.**

1.6 On day 2, carefully remove 700 µl of the culture medium I from each well and replace it by fresh culture medium I, to maintain an appropriate GM-CSF concentration

1.7 On day 3, remove and discard the culture medium I completely on day 3. Wash the cells carefully with 500 µl per well using culture medium II to remove residual non adherent cells

1.8 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
1.10 On day 6, harvest the immature dendritic cells by collecting non adherent cells and loosely adherent cells, or mature cells as described below. To release loosely adherent cells, wash off the cells thoroughly by pipetting them with culture medium I. Discard adherent cells
1.11 Continue with Nucleofection® as described in chapter 2

**Maturation of dendritic cells**

1.12 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 (after step 1.9 of the isolation procedure described above) and replace it by 1 ml maturation media
1.13 Incubate the cells for one more day
1.14 On day 7, harvest the mature dendritic cells 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 adherent cells
1.15 Continue with Nucleofection® as described in chapter 2

## 2. Nucleofection®

**One Nucleofection® Sample contains**

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| 2.5 x $10^5$ cells |
| 2 µg plasmid DNA (in 1 – 5 µl $H_2O$ or TE) or 2 µg pmaxGFP® Vector or 30 – 300nM siRNA (3 – 30 pmol/sample) |
| 100 µl Mouse Dendritic Nucleofector® Solution |

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare 48-well plates by filling appropriate number of wells with 0.4 ml of supplemented culture medium I 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 (**2.5 x $10^5$ cells per sample**) **at 300xg for 10 minutes** at room temperature. Discard supernatant completely so that no residual medium 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 minutes in Mouse Dendritic Cell Nucleofector® Solution, as this reduces cell viability and gene transfer efficiency
2.6 Combine 100 µl of cell suspension with **2 µg DNA,** 2 µg pmaxGFP® Vector or **30 – 300 nM** siRNA (3 – 30 pmol/sample) 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 **Y-001** (for immature dendritic cells) or **AN-001** (for mature dendritic cells)
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 For mature cells only: Incubate cuvette for 10 minutes at room temperature

2.12 Add ~400 µl of the pre-equilibrated culture medium I to the cuvette and gently transfer the sample into the 48-well plate (final volume of 0.8 ml media per well). 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. If this is not the case, the incubation period may be prolonged to 24 hours

## 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:**

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: scientific.support@lonza.com

Europe and Rest of World
Phone: +49 221 99199 400
Fax: +49 221 99199 499
E-mail: scientific.support.eu@lonza.com

### References

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

**Lonza Cologne AG**
**50829 Cologne, Germany**



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