

# Amaxa™ Basic Nucleofector™ Kit for Primary Mammalian Endothelial Cells

## For Primary Mammalian Endothelial Cells

Cells derived from mammalian endothelial tissues from various organs; large and flat adherent cells, often with large nuclei.

**Note** Mammalian endothelial cells display significant phenotypic variations due to the wide range of both species and body sites from which they may be sourced. You can determine the optimal Nucleofection™ condition for your endothelial cells using the Basic Nucleofector™ Kit for Primary Mammalian Endothelial Cells [Cat. No. VPI-1001]. Please find some guidelines on endothelial cell culture for Nucleofection™ and on the transfection procedure using our Basic Nucleofector™ Kit below. However, we recommend referring to more detailed culture protocols before you start the experiments. Having tested various endothelial cell types, high transfection efficiencies could be achieved using one of the programs indicated below. If you do not attain satisfying results with your endothelial cells of interest, please contact our Scientific Support Team for further help with the optimization. On our website ([www.lonzabio.com](http://www.lonzabio.com)) we provide a form you might use to enter the results achieved with the Basic Kit.

## Product Description

|  |  |
|--|--|
| Cat. No.   | VPI-1001   |
| Size (reactions)   | 25   |
| Basic Nucleofector™ Solution for Mammalian Endothelial Cells | 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. |

## Optimization Guidelines

The initial optimization experiment is comprised of 6 reactions: 5 different Nucleofector™ Programs are tested with 1 Nucleofector™ Solution plus 1 control (no program). The Nucleofector™ Program which turns out to be the most appropriate should be used for all subsequent transfections.

A further fine tuning of the Nucleofection™ condition can be performed with the help of our Scientific Support Team.

|         | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6   |
|---------|----------|----------|----------|----------|----------|------------|
| Program | M-003    | T-005    | T-023    | T-027    | U-011    | No program |

## Basic Nucleofector™ Protocol for Primary Mammalian Endothelial Cells

The Basic Nucleofector™ Kit for Primary Endothelial Cells has been tested successfully for the following cell types provided by Lonza:

| Cell type  | Lonza Cat. No. | Optimal Nucleofector™ Program | Transfection efficiency | Viability (Vialight® Plus Kit, Lonza) |
|--|----------------|-------------------------------|-------------------------|---------------------------------------|
| HAEC (Human Aortic Endothelial Cells)                              | CC-2535        | M-003                         | 59%                     | 45-60%                                |
| HMVEC-dAd (Adult Human Dermal Microvascular Endothelial Cells)     | CC-2543        | M-003                         | 63%                     | 40-50%                                |
| HMVEC-dNeo (Neonatal Human Dermal Microvascular Endothelial Cells) | CC-2516        | M-003                         | 61%                     | 55-60%                                |

### 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
- Supplied pmaxGFP™ vector
- Substrate of interest, highly purified, preferably by using endotoxin free kits; A260 : A280 ratio should be at least 1.8
- Culture dishes (Ø6 cm/sample) or culture system of your choice
- **For trypsinization:** Please use trypsin as recommended by the cell supplier e.g. Reagent Pack™ Subculture Reagent Kit containing Trypsin/EDTA, HEPES Buffered Saline Solution (HBSS) and Trypsin Neutralizing Solution (TNS) [Lonza, Cat. No. CC-5034]. Alternatively if cells hardly detach: Trypsin 0.5%-EDTA 0.2%
- **Culture medium:** Please use a medium especially suited for the culture of primary endothelial cells, e.g. Endothelial Cell Medium, EGM®-2 with BulletKit® Additives [Lonza, Cat. No. CC-3162] or a different special medium recommended for your endothelial cell type containing all required supplements. We recommend storing 40 ml aliquots of the prepared medium at -80°C. Do not use medium stored at 4°C for more than two days, as this may lead to reduced cell viability and transfection efficiency
- Prewarm appropriate volume of culture medium to 37°C (3 ml per sample)
- Appropriate number of cells (4–5 x 10<sup>5</sup> cells per sample)  
Minimal cell number: 2 x 10<sup>5</sup> cells (a lower cell number may lead to a major increase in cell mortality)  
Maximum cell number: 7 x 10<sup>5</sup>

## 1. Pre Nucleofection™

**Note** Transfection results may be source-dependent. Culture conditions may differ between cell types. Please follow your established procedure or the supplier's recommendations.

### Cell culture recommendations

- 1.1 Replace medium every 2 days (6–7 ml per 75 cm<sup>2</sup> flask)
- 1.2 Cells should be passaged after reaching 75 % confluency
- 1.3 Do not use cells after passage 9 for Nucleofection™
- 1.4 Cells should be passaged 3–5 days before Nucleofection™
- 1.5 Optimal confluency before Nucleofection™ 70–80%; higher confluency may reduce cell viability

### Trypsinization

- 1.6 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
- 1.7 For harvesting, incubate the cells up to 10–15 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
- 1.8 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached

## 2. Nucleofection™

### One Nucleofection™ sample contains

|   |
|---|
| 4–5 x 10 <sup>5</sup> cells   |
| 1–3 µg plasmid DNA (in 1–5 µl H <sub>2</sub> O or TE) or 2 µg pmaxGFP™ vector or 30–300 nM siRNA (3–30 pmol/sample) |
| 100 µl Basic Nucleofector™ Solution for Mammalian Endothelial Cells   |

- 2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
- 2.2 Prepare culture dishes (Ø6 cm) by filling appropriate number of wells with 2.5 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO<sub>2</sub> incubator
- 2.3 Harvest the cells by trypsinization (please see 1.6–1.8)
- 2.4 Count an aliquot of the trypsinized cells and determine cell density
- 2.5 Centrifuge the required number of cells (4–5 x 10<sup>5</sup> cells per sample) at 100xg for 10 minutes at room temperature
- 2.6 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector™ Solution per sample
- 2.7 Combine 100 µl of cell suspension with 1–3 µg DNA, 2 µg pmaxGFP™ vector (recommended for initial optimization), or 30 nM–300 nM siRNA (3–30 pmol/sample) or other substrates
- 2.8 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap
- 2.9 Select the appropriate Nucleofector™ Program. Please try all 5 Nucleofector™ Programs initially to determine the most appropriate one for your smooth muscle cell type for all subsequent experiments M-003, T-005, T-023, T-027, U-011 (M-03, T-05, T-23, T-27 and U-11 for Nucleofector™ I Device)

- 2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector™ Cuvette Holder and apply the selected program
- 2.11 Take the cuvette out of the holder once the program is finished
- 2.12 Add ~500 µl of the pre-equilibrated culture media to the cuvette and gently transfer the sample immediately into the culture dish (Ø6 cm) [final volume 3 ml media per well]. Use the supplied pipettes and avoid repeated aspiration of the sample

### 3. Post Nucleofection™

- 3.1 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 but ideally, cells should be left undisturbed for 24 hours

### Additional Information

For an up-to-date list of all primary endothelial cells successfully transfected with this Basic Nucleofector™ Kit, please refer to:

[www.lonza.com/cell-database](http://www.lonza.com/cell-database)

For an up-to-date list of all Nucleofector™ References, please refer to:

[www.lonza.com/nucleofection-citations](http://www.lonza.com/nucleofection-citations)

For more technical assistance, contact our Scientific Support Team:

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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.

The Nucleofector™ technology, comprising Nucleofection™ process, Nucleofector™ device, Nucleofector™ solutions, Nucleofector™ 96-well Shuttle™ System and 96-well Nucleocuvette™ plates and modules is covered by patent and/or patent-pending rights owned by Lonza Cologne GmbH.

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