

# Amaxa™ 96-well Shuttle™ Basic Protocol for Primary Mammalian Endothelial Cells

## Cell Description

Large, flat adherent cells derived from mammalian endothelial cell tissues from various organs.

### Note

Mammalian endothelial cells display significant phenotypic variations due to the wide range of both species and tissues from which they may be sourced.

This basic protocol describes how to easily define optimal Nucleofection™ Conditions for different mammalian endothelial cells. We recommend to first test a set of pre-selected Nucleofector™ Programs together with the P5 Primary Cell 96-well Nucleofector™ Kit.

If you have questions regarding your endothelial cells of interest, please contact our Scientific Support Team for further help with the optimization.

## Product Description

### Recommended Kits

P5 Primary Cell 96-well Nucleofector™ Kits

Cat. No.	V4SP-5096
Size (reactions)	1×96
P5 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	1

Cat. No.	V4SP-5960
Size (reactions)	10×96
P5 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5.0 ml
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg
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.

### Note

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. They are not compatible with the Nucleofector™ II/2b Device.

## Optimization Guidelines

The initial optimization experiment is comprised of 16 reactions, using 1 Nucleocuvette™ Module: 7 different Nucleofector™ Programs are tested in duplicate plus 1 control. The program, which turned out to be the most appropriate Nucleofection™ Condition, should be used for all subsequent transfections.

5×10 <sup>4</sup> –1×10 <sup>5</sup> cells/sample		
1	2	3–12
A 96-CA-167	96-CA-167	—
B 96-DY-138	96-DY-138	—
C 96-EH-100	96-EH-100	—
D 96-EP-114	96-EP-114	—
E 96-FA-100	96-FA-100	—
F 96-FF-138	96-FF-138	—
G 96-FP-100	96-FP-100	—
H negative control (no program)	negative control (no program)	—

The P5 Primary Cell 96-well Nucleofector™ Solution has been tested successfully for the following cell types provided by Lonza:

Cell type	Lonza Cat. No.	Optimal Program	Transfection efficiency	Viability*
HAEC (Human Aortic Endothelial Cells)	CC-2535	96-EH-100	73 %	70 %
HMVEC-L (Human Lung Microvascular Endothelial Cells)	CC-2527	96-FP-100	79 %	48 %

\*Determined by ViaLight™ Plus Kit, Lonza

## Required Material

### Note

Please make sure that the entire supplement is added to the Nucleofector™ Solution.

- Nucleofector™ 96-well Shuttle™ System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature prior to Nucleofection™
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µl
- Supplied Nucleocuvette™ Plates

### 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
- Nucleocuvette™ 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
- For trypsinization: Please use trypsin as recommended by the cell supplier e.g. ReagentPack™ Subculture Reagent Kit containing Trypsin/EDTA, HEPES Buffered Saline Solution (HEPES-BSS) and Trypsin Neutralizing Solution (TNS) [Lonza; Cat. No. CC-5034]
- Culture medium: please use media as recommended by the cell supplier e.g. EGM™-2 BulletKit™ [Lonza; Cat. No. CC-3162] for HAEC [Lonza; Cat. No. CC-2535] and HUVEC [Lonza; Cat. No. CC-2517] or EGM™-2 MV BulletKit™ [Lonza; Cat. No. CC-3202] for HMVEC-L [Lonza; Cat. No. CC-2527]
- Prewarm appropriate volume of culture medium to 37°C (150 µl per sample)
- Appropriate number of cells (5 × 10<sup>4</sup>–1 × 10<sup>5</sup> cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection™

### Note

Transfection results may vary due to different culture conditions prior and post Nucleofection™. Especially endothelial cells show a dramatic decrease of transfection efficiency, if cells were grown confluent during subculture procedure (contact inhibition) 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 2–3 days
- 1.2 Cells should be passaged after reaching 70–80% confluency
- 1.3 Do not use cells after passage 9 for Nucleofection™ as this may lead to reduced viabilities and transfection efficiencies
- 1.4 Cells should be passaged 2–5 days before Nucleofection™ depending on growth rate of cells

### Trypsinization

#### Note

Please follow your established procedure or the supplier's recommendations (e.g. for Human Aortic Endothelial Cells [Lonza; Cat. No. CC-2535] follow procedure described below).

- 1.5 Remove media from the cultured cells and wash cells once with HEPES-BSS
- 1.6 For harvesting, incubate the cells ~5 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
- 1.7 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached

## 2. Nucleofection™

### One Nucleofection™ Sample Contains

- 5 × 10<sup>4</sup>–1 × 10<sup>5</sup> cells
  - 0.4 µg pmaxGFP™ Vector or 0.4 µg plasmid DNA (in 1–2 µl H<sub>2</sub>O or TE) or 30–300nM siRNA (0.6–6 pmol/sample)
  - 20 µl P5 Primary Cell 96-well Nucleofector™ Solution
- 2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
  - 2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details: See device and software manuals)
  - 2.3 Select appropriate Nucleofector™ Program. Please try all 7 Nucleofector™ Programs (96-CA-167, 96-DY-138, 96-EH-100, 96-EP-114, 96-FA-100, 96-FF-138 and 96-FP-100) initially to determine the most appropriate Nucleofection™ Condition for your specific endothelial cell type

- 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 70 µl (see note at the end of this chapter) for one well of a 96-well plate and preincubate/equilibrate plates in a humidified 37°C/5% CO<sub>2</sub> incubator
- 2.5 Pre-warm an aliquot of culture media to 37°C (80 µl per sample\*)
- 2.6 Prepare 0.4 µg pmaxGFP™ Vector (recommended for initial optimization) or 0.4 µg plasmid DNA. For siRNA experiments we recommend to start using 30–300 nM siRNA (0.6–6 pmol/sample). Depending on cell type, the minimum effective siRNA concentration can range between 1 nM and 1 µM. To validate optimal conditions for down-regulation we recommend performing a time course (mRNA: 12–72 hours, protein/phenotype: 24–96 hours) in addition
- 2.7 Harvest the cells by trypsinization (please see 1.5–1.7)
- 2.8 Count an aliquot of the trypsinized cells and determine cell density
- 2.9 Centrifuge the required number of cells (5×10<sup>4</sup>–1×10<sup>5</sup> cells per sample) at 90×g for 10 minutes at room temperature
- 2.10 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofactor™ 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 96-well Nucleocuvette™ Modules

**B: Multiple substrates (e.g. Library Transfection)**

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

**Note**

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well Nucleofactor™ 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.

- 2.11 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well
- 2.12 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well “A1” must be in upper left position

- 2.13 Start 96-well Nucleofection™ Process by either pressing “Upload and start” in the 96-well Shuttle™ Software or pressing “Upload” in the 96-well Shuttle™ Software and then the “Start” button at the 96-well Shuttle™ (for both options please refer to the respective Manual)
- 2.14 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer
- 2.15 Resuspend cells with 80 µl\* (recommendation for 96-well plates) or desired volume of pre-warmed medium (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times. Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 30 µl (if 1×10<sup>5</sup> cells/sample were transfected by Nucleofection™; if lower cell numbers were used, please adjust the appropriate volume) of resuspended cells to 70 µl pre-warmed media prepared in 96-well culture plates\*

**\* Note**

The indicated cell numbers and volumes produce optimal 96-well Nucleofection™ Results in most cases. However, you may wish to test an extended range of cell numbers depending on your specific needs. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

### 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. To validate optimal conditions for down regulation we recommend performing a time course (please see 2.6)

## Additional Information

### Up-To-Date List of all Nucleofector™ References

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

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