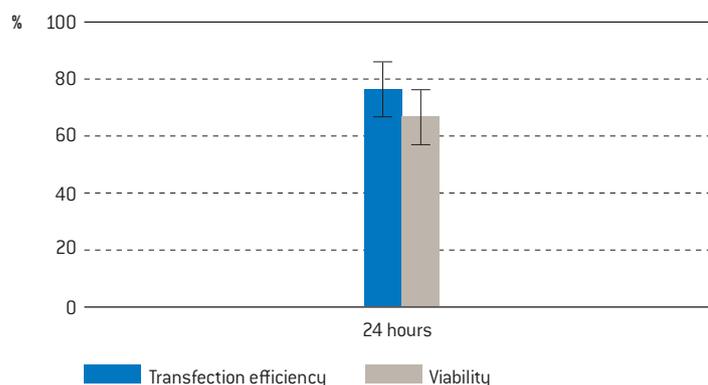


# Amaxa™ 96-well Shuttle™ Protocol for Human Umbilical Vein Endothelial Cells (HUVEC)

## Cell Description

This protocol has been validated for Clonetics™ HUVEC (e.g. Lonza; Cat. No. CC-2519) or self isolated HUVEC; large flat adherent epitheloid cells with large nuclei; cells may grow in confluent monolayer.

### Example for 96-well Nucleofection™ of HUVECs



**Average transfection efficiency and viability of HUVEC 24 hours post Nucleofection™.** HUVECs were transfected with program 96-CA-167 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 determined as % PI negative cells compared to untreated.

## 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 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 ml
pmaxGFP™ Vector [1 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 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.

### 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.

## 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
- Supplied Nucleocuvette™ Plate(s)
- 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
- 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.

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260 : A280 ratio should be at least 1.8
- 96-well culture plates or culture plates of your choice
- For trypsinization: Reagent Pack™ Subculture Reagent Kit containing Trypsin/EDTA, HEPES Buffered Saline Solution (HBSS) and Trypsin Neutralizing Solution (TNS) (Lonza, Cat. No. CC-5034)
- Culture medium: EGM™-2 BulletKit (Lonza; Cat. No. CC-3162)). 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 (255 µl per sample)
- Appropriate number of cells (1×10<sup>5</sup> cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection™

### Note

Transfection results may be donor dependent.

### Cell Culture Recommendations

- 1.1 Seeding conditions: 5–6×10<sup>4</sup> cells per 25 cm<sup>2</sup> flask
- 1.2 Replace media 2–3 times per week; 2–3 ml media per 25 cm<sup>2</sup> flask
- 1.3 Cells should be passaged after reaching 80–90 % confluency
- 1.4 For Nucleofection™ cells should be preferably passaged 2 days before
- 1.5 Do not use cells after passage number 10 as this may result in substantially lower gene transfer efficiency and viability
- 1.6 Optimal confluency before Nucleofection™: 90 %

### Trypsinization

- 1.7 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
- 1.8 For harvesting, incubate the cells ~1–3 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material). If necessary, prolong the incubation time for two more minutes at 37°C
- 1.9 Neutralize trypsinization reaction with TNS once the majority of the cells (>90 %) have been detached

## 2. Nucleofection™

### One Nucleofection™ Sample Contains

- $1 \times 10^5$  cells
- 0.1–1 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 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 the appropriate 96-well Nucleofector™ Program **96-CA-167**

### Note

For self-isolated HUVEC we recommend testing the programs suggested in our Basic Protocol for Primary Mammalian Endothelial Cells in addition to 96-CA-167 (96-DY-138, 96-EH-100, 96-EP-114, 96-FA-100, 96-FF-138 and 96-FP-100)

- 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 175 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 medium to 37°C (80 µl per sample\*)
- 2.6 Prepare 0.1–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 2.7 Harvest the cells by trypsinization (please see 1.7–1.9)
- 2.8 Count an aliquot of the cells and determine cell density
- 2.9 Centrifuge the required number of cells ( $1 \times 10^5$  cells per sample) at 90×g for 10 minutes at room temperature. Remove supernatant completely
- 2.10 Resuspend the cell pellet carefully in 20 µl room temperature 96-well 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 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 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.

- 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
- 2.16 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 25 µl of resuspended cells to 175 µl pre-warmed medium prepared in 96-well culture plates\*

### \* Note

The indicated cell numbers and volumes have been found to produce optimal 96-well 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.

## 3. Post Nucleofection™

- 3.1 Incubate the cells in 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

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