

HUVEC Nucleofector® Kit

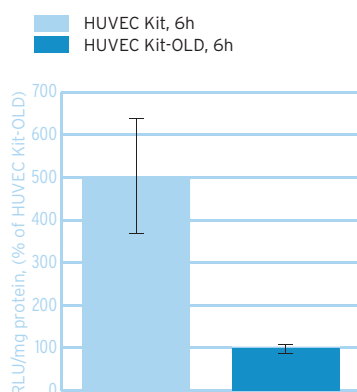
Human Umbilical Vein Endothelial Cells (HUVEC)

[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

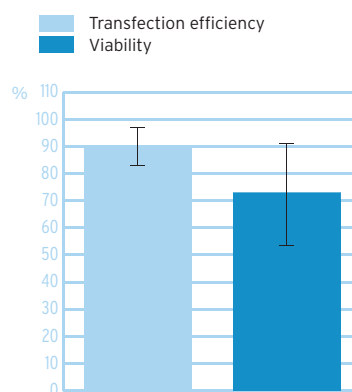
Note !

There are two different kits for Nucleofection® of HUVECs available: HUVEC Nucleofector® Kit [Cat.No. VPB-1002] and HUVEC Nucleofector® Kit-OLD [Cat.No. VPB-1492]. The HUVEC Nucleofector® Kit offers better transfection efficiencies and enhanced protein expression.

Major improvement of protein expression with the HUVEC Nucleofector® Kit



Primary HUVEC [Lonza] were transfected using the HUVEC Nucleofector® Kit or the HUVEC Nucleofector® Kit-OLD with 2 µg of a plasmid encoding firefly luciferase. 6 hours post Nucleofection® cells were lysed and luciferase expression was measured with a microplate reader using Steady-Glo™ reagent [Promega]. Values were normalized to protein content of the lysates and expressed as percentage of the value with the HUVEC Nucleofector® Kit-OLD. A 5-fold increase in protein expression can be achieved with the improved HUVEC Nucleofector® Kit.



Transfection efficiency and viability of HUVEC [Lonza] 24 hours post Nucleofection®. Cells were transfected with Nucleofector® program A-034 and 2 µg of pmaxGFP®, 24 hours post Nucleofection® cells were analyzed by flow cytometry.

Product Description

Cat. No.

VPB-1002

2.25 ml HUVEC Nucleofector® Solution
0.5 ml Supplement
10 µg pmaxGFP® (0.5 µg/µl in 10 mM Tris pH 8.0)
25 certified cuvettes
25 plastic pipettes

Size

25 reactions

Storage and stability

Store Nucleofector® Solution, Supplement and pmaxGFP® at 4°C. For long term storage pmaxGFP® 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.

Required Material

Note !

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

- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- 6-well culture dish or culture system 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]
- Appropriate volume of culture media at 37°C (1.5 ml per sample; 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**
- Supplied pmaxGFP® control DNA
- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260:A280 ratio should be at least 1.8
- Nucleofector® device
- Appropriate number of cells (5 x 10⁵ cells per sample)
Minimal cell number: 5 x 10⁴ cells (a lower cell number may decrease cell viability)
Maximum cell number: 1 x 10⁶ cells

1. Pre Nucleofection®

Note !

Transfection results may be donor-dependent.

Cell samples

- 1.1 Human Umbilical Vein Endothelial Cells (HUVEC; cryopreserved) from Lonza [Cat. No. CC-2519] or self isolated HUVECs

Cell culture recommendations

- 1.2 Seeding conditions: 5-6 x 10⁴ cells per 25 cm² flask
- 1.3 Replace media 2-3 times per week; 2-3 ml media per 25 cm² flask
- 1.4 Cells should be passaged after reaching 80-90% confluency
- 1.5 For Nucleofection® cells should be preferably passaged 2 days before
- 1.6 Do not use cells after passage number 10 as this may result in substantially lower gene transfer efficiency and viability
- 1.7 Optimal confluency before Nucleofection® 90%

Trypsinization

- 1.8 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
- 1.9 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
- 1.10 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached

2. Nucleofection®

Note ! HUVECs are sensitive to prolonged incubation in HUVEC Nucleofector® Solution. We therefore recommend processing a maximum of 5 samples in parallel to keep incubation time at a maximum of 5 minutes (average time per sample is 1 minute).

Note ! When using self isolated HUVECs we recommend testing two Nucleofector® programs: A-034 and U-001 in parallel, as U-001 has shown sometimes higher transfection efficiency and/or viability. For HUVECs from Lonza we always recommend using program A-034 only.

- 2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution!
- 2.2 Prepare 6-well plates by filling appropriate number of wells with 1.5 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO₂ incubator
- 2.3 Harvest the cells by trypsinization (please see 1.8-1.10)
- 2.4 Count an aliquot of the trypsinized cells and determine cell density
- 2.5 Centrifuge the required number of cells (**5 x 10⁵ cells per sample**) at **200xg 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 **0.5-5 µg DNA, 2 µg pmaxGFP®** or appropriate amount of **siRNA (30 nM - 300 nM** or 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

One Nucleofection® sample contains

- › 5 x 10⁵ cells
- › 0.5-5 µg plasmid DNA (in 1-5 µl H₂O or TE) or 2 µg pmaxGFP® or 30-300 nM siRNA (3-30 pmol/sample)
- › 100 µl Nucleofector® Solution

- 2.9 Select the appropriate Nucleofector® program
A-034 for HUVECs from Lonza or
U-001 additionally for self isolated HUVECs
- 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 6-well plate (final volume 1.5 ml media per well/sample). 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%, CO₂ incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4-8 hours

Additional Information

Recent Publications

Zenner HL et al., J Cell Sci. 2007; 120(Pt12):2117-2125

Sprenger RR et al., Biochem J (2006) 400(3): 401-10

Gong R et al., Kidney Int (2006) 69(7): 1166-74

Opitz B et al., J Immunol (2006) 176(1): 484-490

For an up-to-date list of all Nucleofector® references, please refer to:

www.amaxa.com/citations

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