› **Optimized Protocol** for Human Umbilical Vein Endothelial Cells (HUVEC)

DPB-1004 Vs. 08-2008

HUVEC Nucleofector® Kit-OLD

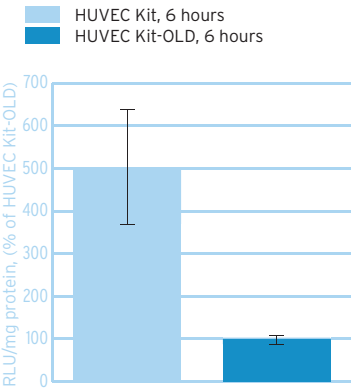
Human Umbilical Vein Endothelial Cells (HUVEC)

[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

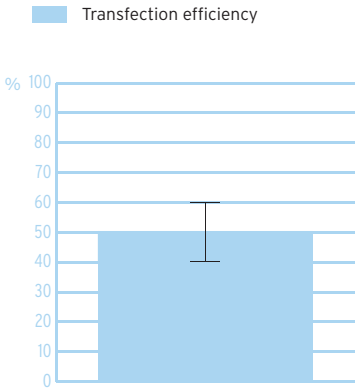
Note !

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 of HUVEC [Lonza] 20-24 hours post Nucleofection®. Cells were transfected using the HUVEC Nucleofector® Kit-OLD, program U-001 and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K^k.

Product Description

Cat. No.	**VPB-1492** 2.25 ml HUVEC Nucleofector® Solution-OLD 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.

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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 (0.5-1 x 10^6 cells per sample)
Minimal cell number: 2 x 10^5 cells (a lower cell number may lead to a major increase in cell mortality)

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 10^4 cells per 25 cm^2 flask
1.3 Replace media 2-3 times per week; 2-3 ml media per 25 cm^2 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-OLD. 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).

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% CO_2 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 (**0.5-1 x 10^6 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 **1-5 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

› 0.5 -1 x 10^6 cells
› 1-5 µg plasmid DNA (in 1-5 µl H_2O or TE) or 2 µg pmaxGFP® or 30-300 nM siRNA (3-30 pmol/sample)
› 100 µl Nucleofector® Solution

2.9 Select appropriate Nucleofector® program
U-001

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% CO_2 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., Bochem 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

For more technical assistance contact our Scientific Support Team:

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