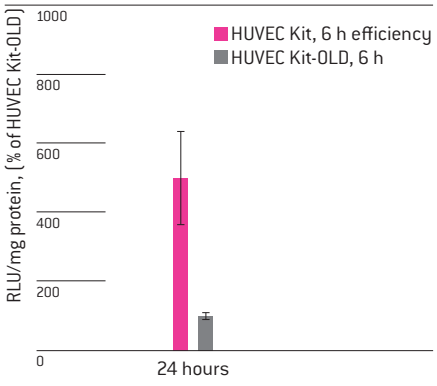
Amaxa® HUVEC Nucleofector® Kit

For Human Umbilical Vein Endothelial Cells (HUVEC)

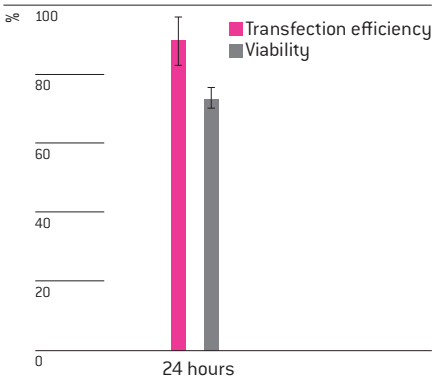
Validated to work with Clonetics® HUVEC (e.g. Lonza; Cat. No. CC-2519) or self isolated HUVEC; large flat adherent epitheloid cells with large nuclei; cells may grow in confluent monolayer

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® Vector. 24 hours post Nucleofection® cells were analyzed by flow cytometry.

Product Description

Cat. No.	**VPB-1002**
Size (Reactions)	25
HUVEC Nucleofector® Solution	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.

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
- 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]
- **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 (1.5 ml per sample)
- Appropriate number of cells (5 x 10^5 cells per sample)
  Minimal cell number: 5 x 10^4 cells (a lower cell number may decrease cell viability)
  Maximum cell number: 1 x 10^6 cells

1. Pre Nucleofection®

Note **Transfection results may be donor – dependent.**

Cell culture recommendations

1.1 Seeding conditions: 5 – 6 x 10^4 cells per 25 cm^2 flask
1.2 Replace media 2 – 3 times per week; 2 – 3 ml media per 25 cm^2 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

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

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 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% CO_2 incubator
2.3 Harvest the cells by trypsinization (please see 1.7 – 1.9)
2.4 Count an aliquot of the trypsinized cells and determine cell density
2.5 Centrifuge the required number of cells (**5 x 10^5 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® Vector 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 **A-034** for HUVECs from Lonza or **U-001** additionally for self isolated HUVECs (A-34 or U-01 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 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 humidified 37°C/5% CO_2 incubator until analysis. Gene expression is often detectable after only 4 – 8 hours

Additional Information

For an up-to-date list of all Nucleofector® References, please refer to:
www.lonza.com/nucleofection-citations

For more technical assistance, contact our Scientific Support Team:

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References

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