

HUVEC Nucleofector® Kit-OLD

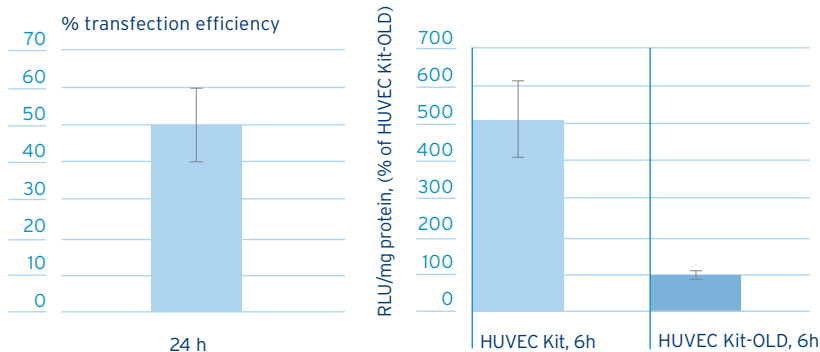
for Human Umbilical Vein Endothelial Cells

Cell type	Origin	Human Umbilical Vein Endothelial Cells (HUVEC) pooled, first passage [e. g. Cambrex; Cat. No. CC-2519 or self-isolated HUVEC].
	Morphology	Large and flat epithelioid cells with large nuclei. Cells may grow in confluent monolayer.

Note

The HUVEC Nucleofector® Kit has recently undergone major improvements. The new HUVEC Nucleofector Kit offers better transfection efficiencies and enhanced protein expression. The kit can be ordered with the Cat. No.: VPB-1002.

Major improvement of protein expression with the HUVEC Nucleofector® Kit.



Transfection efficiencies of HUVEC [Cambrex] 20-24 hours post nucleofection. Cells were nucleofected using the HUVEC Nucleofector® Kit-OLD, program **U-01/U-001** and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K^k.

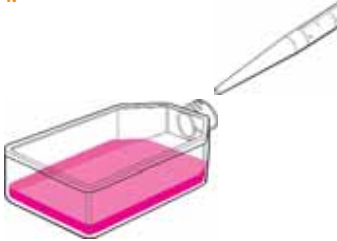
Primary HUVEC [Cambrex] were nucleofected using the HUVEC Nucleofector® Kit or the HUVEC Nucleofector® Kit-OLD with 2 µg of a plasmid encoding firefly luciferase. 6h post nucleofection cells were lysed and luciferase expression was measured with a microplate reader using Steady-Glo™ reagent (Promega). 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.

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Procedure outline & important advice

1.



Procedure outline

Culturing of cells before nucleofection.
(For details see 3.1. and 3.3.)

Important advice

- › Use EGM-2 BulletKit (stored < 2d at 4°C).
- › Follow passaging instructions.
- › Preferably passage cells 2 days before nucleofection.
- › Passage number <10.
- › Confluency before nucleofection: 90%.
- › Prewarm medium to 37°C.

2.



Combine the cells of interest, DNA or siRNA and the appropriate cell-type specific Nucleofector Solution and transfer to an amaxa certified cuvette.
(For details see 3.5.)

Contents of one nucleofection sample:

- › 0.5-1 x 10⁶ cells (optimal cell number).
- › 1-5 µg highly purified plasmid DNA (in max. 5 µl) or 0.5-3 µg siRNA.
- › 100 µl HUVEC Nucleofector Solution-OLD.

Do not incubate HUVEC longer than 5 min in HUVEC Nucleofector Solution-OLD. It is recommended not to process more than 5 samples at one time.

3.

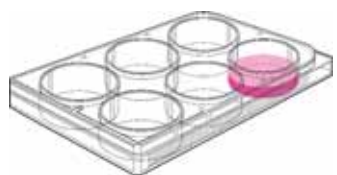


Choose the cell-type specific program. Insert the cuvette into the Nucleofector and press the "X" button.
(For details see 3.5.)

- › Optimal Nucleofector program:
U-01*/U-001**

* for Nucleofector I Device
**for Nucleofector II Device

4.



Rinse the cuvette with pre-warmed culture medium and transfer the cells into the culture dish.
(For details see 3.5.)

- › Gently remove sample from the cuvette with 500 µl pre-warmed culture medium.
- › Transfer directly to 37°C.

2

Product description

Cat. No.	VPB-1492
Kit components	2.25 ml HUVEC Nucleofactor Solution-OLD 0.5 ml Supplement-OLD 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 Nucleofactor Solution and Supplement at 4°C. For long term storage pmaxGFP is ideally stored at -20°C. The expiry date is printed on the Solution Box.

3

Protocol



3.1 › **Required reagents**

Medium EGM-2 BulletKit [Cambrex; Cat. No. CC-3162]. Note: We recommend storing 40 ml aliquots of prepared medium at -80°C. **Do not use medium stored for more than two days at 4°C because this can lead to an increase in cell mortality and reduction of gene transfer efficiency.**

Trypsin treatment We recommend using Reagent Pack™ Subculture Reagent Kit containing trypsin/EDTA, HEPES Buffered Saline (HBS) and Trypsin Neutralizing Solution (TNS). [Cambrex; Cat. No. CC-5034].



3.2 › **DNA preparation and quality**

The quality and the concentration of DNA used for nucleofection plays a central role for the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purification like QIAGEN EndoFree® Plasmid Kits [Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit). The purified DNA should be resuspended in deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 1-5 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio, according to QIAGEN protocol.





3.3 › Cell culture

- Culture conditions** Replace medium 2-3 times per week (2-3 ml medium per 25 cm² flask).
- Passage interval** Cells should be passaged after reaching 80-90% confluency.
- Seeding conditions** At least 5-6 x 10⁴ cells per flask (25 cm²).

Culture conditions before nucleofection

- › The cells should be preferably passaged 2 days before nucleofection.
- › Do not use cells after passage number 10 for nucleofection. Nucleofection of the cells after passage 10 may result in substantially lower gene transfer efficiency and trypsin treatment is more difficult and may damage the cells.
- › Cells should be nucleofected after reaching 90% confluency.

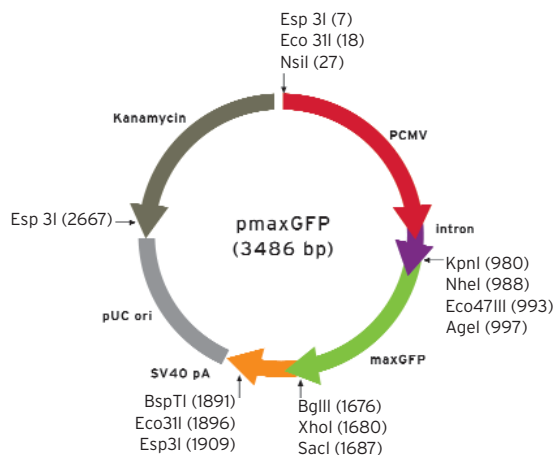
Note

Contamination of cell culture with mycoplasma is a wide spread phenomenon that might negatively influence experimental results. We recommend the use of Primocin™ [Cat. No. VZA-1021], a new antibiotic formulation specifically developed to protect sensitive primary cells from mycoplasma infection and microbial contaminations. Add it directly to the cell culture medium without further need of Pen/Strep or other antibiotics. For more information and ordering info see www.amaxa.com/antibiotics.

3.4 › Important controls and vector information

Positive control

We strongly recommend establishing the Nucleofector technology with the positive control vector **pmaxGFP™** as provided in this kit. pmaxGFP encodes the green fluorescent protein (GFP) from copepod *Pontellina p.* Just like eGFP expressing cells, maxGFP expressing cells can easily be analyzed by fluorescence microscopy or flow cytometry to monitor transfection efficiency.



Negative control

We recommend you always perform two control samples to assess the initial quality of cell culture and the potential influences of nucleofection or amount/purity of DNA on cell viability.

control 1 Recommended amount of cells in Nucleofector solution with DNA but without application of the program (alternatively: untreated cells)
(Cells + Solution + DNA - program)

control 2 Recommended amount of cells in Nucleofector solution without DNA with application of the program **(Cells + Solution - DNA + program)**

Vector information

If using IRES sequences in your vectors, please remember that the gene encoded 3' of the IRES sequence is usually expressed to a lesser extent than the upstream gene, and in some cell types may not be expressed at all. As alternatives we suggest either: co-transfecting two (or more) plasmids, using one plasmid with each gene under the control of its own promoter, or making a GFP fusion.

3.5 › Nucleofection protocol

Preparation of Nucleofector Solution

Add 0.5 ml Supplement to 2.25 ml Nucleofector Solution and mix gently. The Nucleofector Solution is now ready to use and is stable for 3 months at 4°C.
Note the date of addition on the vial.

One nucleofection sample contains

- › **0.5-1 x 10⁶ cells**
- › **1-5 µg plasmid DNA (in 1-5 µl H₂O or TE) or 2 µg pmax GFP or 0.5-3 µg siRNA**
- › **100 µl HUVEC Nucleofector Solution-OLD**

Minimal cell number: 2 x 10⁵ cells (a lower cell number may lead to a major increase in cell mortality).

For more details about the nucleofection of siRNA:
www.amaxa.com/RNAi

Note



HUVEC 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).

Preparation of samples

1. Cultivate the required number of cells.
2. Prepare **1-5 µg DNA** or **0.5-3 µg siRNA** for each sample.
3. Pre-warm the supplemented HUVEC Nucleofector Solution-OLD to room temperature.

re. Pre-warm an aliquot of culture medium containing serum and supplements at 37°C in a 50 ml tube (500 µl per sample).

4. Prepare 6-well plates by filling the appropriate number of wells with 1 ml of culture medium containing serum and supplements and pre-incubate plates in a humidified 37°C/5% CO₂ incubator.
5. Remove the medium from the tissue culture. Wash cells once with HBS (ReagentPack), using at least the same volume of HBS as culture medium. Aspirate and discard the HBS.
6. Harvest the cells by trypsinization: Add trypsin / EDTA solution (ReagentPack) to cover the cell monolayer, and gently swirl the dish/flask to ensure an even distribution of the solution. Place the dish/flask in an incubator at 37°C until the cells start to detach (usually after 1-3 minutes). Remove the dish/flask from the incubator. Tap against the sides of the dish/flask to improve detachment. Carefully check the process under a microscope to avoid overexposure of cells to trypsin. If necessary, prolong the incubation time for two more minutes at 37°C. Once the majority of cells (>90%) have been dislodged, add TNS (ReagentPack) to inactivate trypsin. Gently resuspend and remove the cells from the flasks by pipetting.
7. Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.
8. Centrifuge the required number of cells (**0.5-1 x 10⁶** cells per nucleofection sample) at **200xg for 10 min**. Discard supernatant completely so that no residual medium covers the cell pellet.
9. Resuspend the pellet in room temperature HUVEC Nucleofector Solution-OLD to a final concentration of **0.5-1 x 10⁶ cells/100 µl**. Avoid storing the cell suspension longer than **15 min** in HUVEC Nucleofector Solution-OLD, as this reduces cell viability and gene transfer efficiency.

Important: Steps 10-14 should be performed for each sample separately.

10. Mix 100 µl of cell suspension with **1-5 µg** DNA or **0.5-3 µg** siRNA.
11. Transfer the nucleofection sample into an amaxa certified cuvette. Make sure that the sample covers the bottom of the cuvette, avoid air bubbles while pipetting. Close the cuvette with the blue cap.
12. Select the Nucleofector program **U-01/U-001** (see Nucleofector Manual for details). Insert the cuvette into the cuvette holder (Nucleofector I : rotate carousel to final position) and press the "X" button to start the program.
13. **To avoid damage to the cells remove the sample from the cuvette immediately after the program has finished** (display showing "OK"). Take the cuvette out of the holder. To transfer the cells from the cuvettes, we strongly recommend using the plastic pipettes provided in the kit to prevent damage and loss of cells. Add 500 µl of the pre-warmed culture medium, containing serum and supplements, and transfer the sample into the prepared 6-well plates. Alternatively, transfer the sample into a 1.5 ml microcentrifuge tube and place it in a 37°C heat block.
14. Press any key to reset the Nucleofector.



Nucleofection



**Cultivation
post nucleofection**

15. Repeat steps 10-14 for the remaining samples.
16. If you have incubated the samples in 1.5 ml microcentrifuge tubes transfer them into the prepared 6-well plates.
17. Incubate cells in a humidified 37°C/5% CO₂ incubator. After 2 hours of incubation viability of cells can be evaluated by proportion of cells attached to the culture wells. Depending on the gene, expression is often detectable after 4-5 hours. If this is not the case, the incubation period may be prolonged.

4

Recommended literature

**Nucleofector
references**

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

www.amaxa.com/citations

* amaxa's Nucleofector® process, Nucleofector® device and Nucleofector® Solutions are covered by PCT applications PCT/EP01/07348, PCT/DE02/01489, PCT/DE02/01483 and other pending patents and domestic or foreign applications corresponding thereto.

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