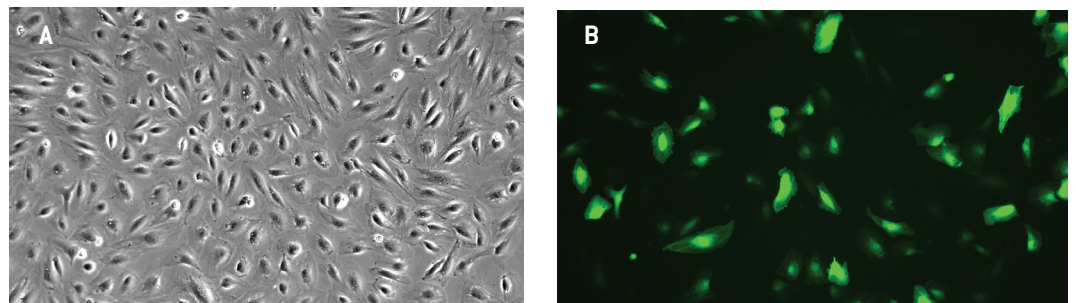


Amaxa® HMVEC-L Nucleofector® Kit

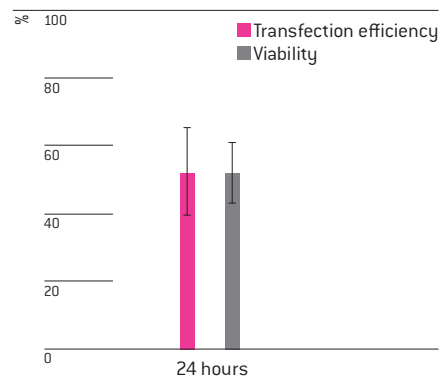
For Human Microvascular Endothelial Cells – Lung (HMVEC-L)

Validated to work with Clonetics® HMVEC-L [Lonza; Cat. No. CC-2527]; adherent endothelial cells

Example for Nucleofection® of HMVEC-L



HMVEC-L were transfected using the HMVEC-L Nucleofector® Kit and a plasmid encoding the enhanced green fluorescent protein eGFP. 25 hours post Nucleofection® cells were analyzed by light (A) and fluorescence microscopy (B).



Average transfection efficiency and viability of HMVEC-L 20 – 24 hours post Nucleofection®. Cells were transfected with program S-005 and 5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP..

Product Description

Cat. No.	VPB-1003
Size (Reactions)	25
HMVEC-L 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®-2MV BulletKit® [Lonza; Cat. No. CC-3202]. We recommend storing 40 ml aliquots of prepared medium at -80°C. Do not use medium stored at 4°C for more than 2 days, as this may lead to reduced cell viability and transfection efficiency
- Prewarm appropriate volume of culture medium to 37°C (2 ml per sample)
- Appropriate number of cells (5 x 10⁵ cells per sample)
Minimal cell number: 2 x 10⁵ [a lower cell number may lead to a major increase in cell mortality]
Maximum cell number: 1 x 10⁶

1. Pre Nucleofection®

Note Transfection results may be donor – dependent.

Cell culture recommendations

- 1.1 Seeding conditions: 1.2 – 1.6 x 10⁵ cells per 25 cm² flask
- 1.2 Replace medium 2 – 3 times per week (2 – 3 ml medium per 25 cm² flask)
- 1.3 Cells should be passaged after reaching 70% confluency
- 1.4 Cells should be passaged 3 – 4 days before Nucleofection® depending on growth rate of cells
- 1.5 Do not use cells after passage number 10 as this may result in substantially lower gene transfer efficiency and viability

Trypsinization

- 1.6 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
- 1.7 For harvesting, incubate the cells up to 10 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
- 1.8 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached

2. Nucleofection®

One Nucleofection® Sample contains

5 x 10⁵ cells

1 – 5 µg plasmid DNA (in 1 – 5 µl H₂O or TE) or 2 µg pmaxGFP® Vector or 30 – 300 nM siRNA
(3 – 30 pmol/sample)

100 µl HMVEC-L Nucleofector® Solution

- 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 2 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 1.6 – 1.8)
- 2.4 Count an aliquot of the trypsinized cells and determine cell density
- 2.5 Count the cells and determine cell density
- 2.6 Centrifuge the required number of cells (5 x 10⁵ cells per sample) at 200xg for 10 minutes at room temperature
- 2.7 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample
- 2.8 Combine 100 µl of cell suspension with **1 – 5 µg DNA, 2 µg pmaxGFP® Vector 30 nM – 300 nM siRNA** (3 – 30 pmol/sample) or other substrates
- 2.9 Transfer cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles. Close the cuvette with the cap
- 2.10 Select the appropriate Nucleofector® Program **S-005** (S-05 for Nucleofector® I Device)
- 2.11 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
- 2.12 Take the cuvette out of the holder once the program is finished
- 2.13 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 2 ml media per well). 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

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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