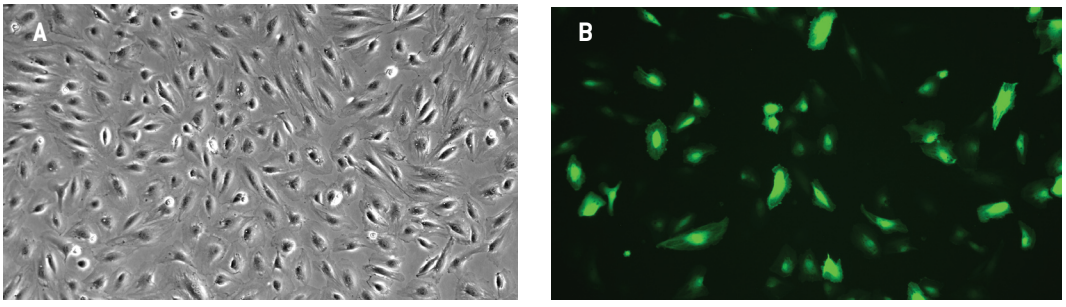
Amaxa® HMVEC-L Nucleofector® Kit

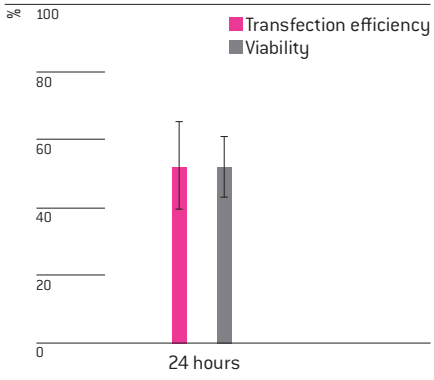
For Human Microvascular Endothelial Cells – Lung (HMVEC-L)

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

1. Pre Nucleofection®

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

Cell culture recommendations

1.1 Seeding conditions: 1.2 – 1.6 x 10^5 cells per 25 cm^2 flask
1.2 Replace medium 2 – 3 times per week (2 – 3 ml medium per 25 cm^2 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 10^5 cells
1 – 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 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% CO_2 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 10^5 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% CO_2 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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