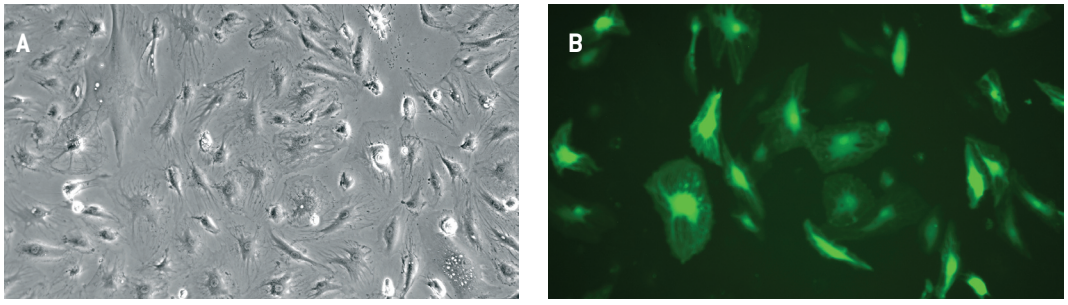
Amaxa® HCAEC Nucleofector® Kit

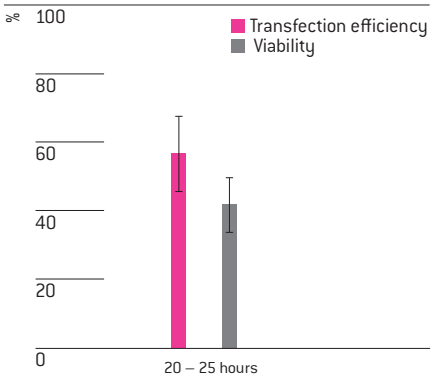
For Human Coronary Artery Endothelial Cells (HCAEC)

Validated to work with Clonetics® HCAEC [Lonza; Cat. No. CC-2585]; large, flat adherent endothelial cells with big nuclei

Example for Nucleofection® of HCAEC



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



Transfection efficiency and viability of HCAEC 20 – 25 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-1001**
Size (reactions)	25
HCAEC 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]. Alternatively if cells hardly detach: Trypsin 0.5 % – EDTA 0.2 %
- **Culture medium:** EGM®-2MV BulletKit® [Lonza; Cat. No. CC-3202]. **We recommend storing 40 ml aliquots of the medium at -80°C. Do not use medium stored for more than two days at 4°C, as this may lead to reduced cell viability and transfection efficiency**
- Prewarm appropriate volume of culture medium to 37°C (2.5 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 major increase in cell mortality)
  Maximum cell number: 5 x 10^5

1. Pre Nucleofection®

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

Cell culture recommendations

1.1 Seeding conditions: 6 – 8 x 10^4 cells per flask (25 cm^2)
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 For Nucleofection® cells should be preferably passaged 3 – 4 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®: 70%

Trypsinization

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

2. Nucleofection®

One Nucleofection® Sample contains

5 x 10^5 cells
1 – 2 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 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 1.7 – 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 **1 – 2 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 **S-005 (S-05 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 2 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

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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DPB-1001_2009-06