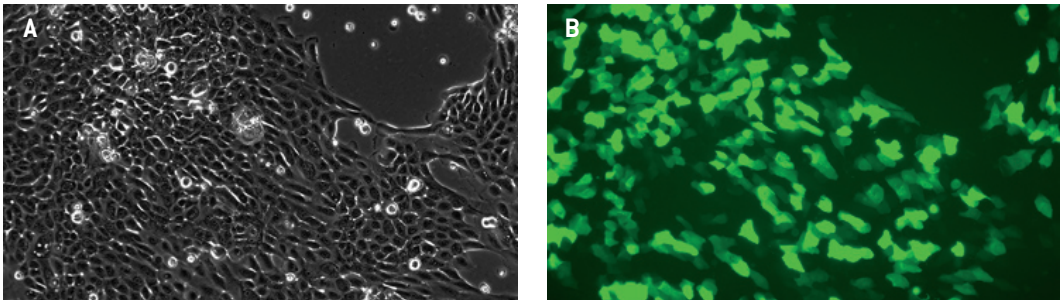
Amaxa® HMEC Nucleofector® Kit

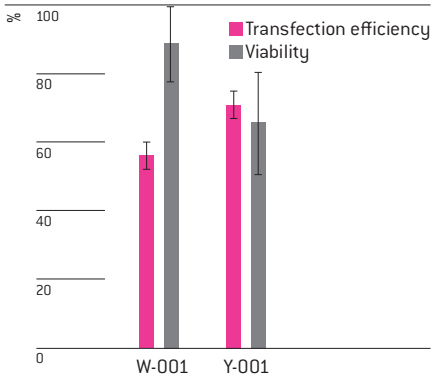
For Human Mammary Epithelial Cells (HMEC)

Validated to work with Clonetics® HMEC [Lonza; Cat. No. CC-2551] ; adherent epithelial cells

Example for Nucleofection® of HMECs



HMECs were transfected with the HMEC Nucleofector® Kit, program Y-001 using a plasmid encoding the green fluorescent protein, maxGFP® Vector. 24 hours post Nucleofection® the cells were analyzed by light (A) or fluorescence microscopy (B).



Transfection efficiency of HMECs 24 hours post Nucleofection®. Cells were transfected with program W-001 or Y-001 and 2 µg of pmaxGFP® Vector. 24 hours post Nucleofection® cells were analyzed by flow cytometry.

Product Description

Cat. No.	**VPK-1002**
Size (reactions)	25
Human Mammary Epithelial Cell (HMEC) 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:** MEGM® BulletKit® [Lonza; Cat. No. CC-3150]. Use 30 ml per 162 cm^2 flask (2 day culture), 40 ml per 162 cm^2 flask (3 day culture). 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 media at 37°C (1.5 ml per reaction)
- Appropriate number of cells (0.5 x 10^6 cells per sample); for lower cell numbers we recommend using program W-001; minimal cell number: 5 x 10^4 cells (viability might decrease) ; maximal cell number: 1 x 10^6 cells

1. Pre Nucleofection®

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

Cell culture recommendations

1.1 Seeding conditions: 2500 cells/cm^2. Use flasks with a surface area of 75 cm^2 only. High cell densities in HMEC culture lead to increased cell mortality and reduced transfection efficiency. This could not be compensated by low density culturing afterwards
1.2 Replace media every 2 – 3 days
1.3 Cells should be passaged every 2 – 3 days
1.4 For Nucleofection® cells should be preferably passaged 2 – 3 days before
1.5 Do not use cells after passage number 14 as this may result in substantially lower gene transfer efficiency and viability
1.6 Optimal confluency before Nucleofection®: 40%

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 ~5 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.9 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached (latest after 7 minutes as otherwise cells may start to clump)

2. Nucleofection®

One Nucleofection® Sample contains

0.5 x 10^6 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 HMEC 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 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 (0.5 x 10^6 cells per sample) **at 200xg for 6 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 **W-001** for high viability or **Y-001** for high transfection efficiency (**W-01** or **Y-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 a 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

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