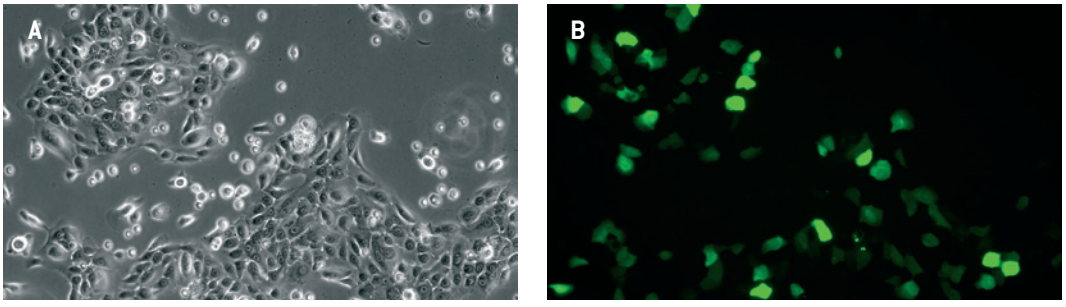
Amaxa™ Nucleofector™ Protocol for Human Prostate Epithelial Cells (PrEC)

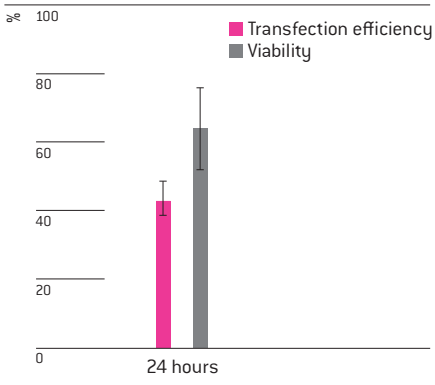
For Human Prostate Epithelial Cells (hPrEC)

Validated to work with Clonetics™ PrEC [Lonza; Cat. No. CC-2555]; adherent epithelial cells

Example for Nucleofection™ of human PrECs



Human PrECs were transfected using program W-001 and 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 hPrECs 24 hours post Nucleofection™. Cells were transfected using program W-001 and 2 µg of pmaxGFP™ Vector. .

Product Description

Recommended Kit(s): Basic Nucleofector™ Kit for Primary Mammalian Epithelial Cells

Cat. No.	**VPI-1005**
Size (reactions)	25
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
- **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]
- Prepared 6-well culture dish
- **Culture medium:** PrEGM® BulletKit [Lonza; Cat. No. CC-3166] per reaction). 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 increased cell mortality and significant reduction of transfection efficiency
- Prewarm appropriate volume of culture media at 37°C (1.5 ml per reaction)
- Appropriate number of cells (5 x 10^5 cells per sample); minimal cell number: 3 x 10^5 cells (a lower cell number may lead to a major increase in cell mortality); maximal cell number: 7 x 10^5 cells

1. Pre Nucleofection™

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

Cell culture recommendations

1.1 Seeding conditions: 2.5 x 10^3 cells/cm^2. Use flasks with a surface area of 75 m^2 only. High cell densities in hPrEC culture lead to reduced cell viability and transfection efficiency
1.2 Replace medium 1 day after splitting, then every 2 days
1.3 Cells should be passaged every 3 – 4 days
1.4 For Nucleofection™ cells should be preferably passaged 2 days before
1.5 Do not use cells after passage number 8 as this may result in substantially lower gene transfer efficiency and viability
1.6 Optimal confluency before Nucleofection™ 60 – 80%

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 4 – 6 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™

Note **When using self-isolated PrECs or cells obtained from another supplier than Lonza we recommend testing the programs of our basic protocol for primary mammalian epithelial cells: S-005, T-013, T-020, W-001, U-017**

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 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 (5 x 10^5 cells per sample) at 220xg for 5 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 – 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** (**W-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 into the 6-well plate. 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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