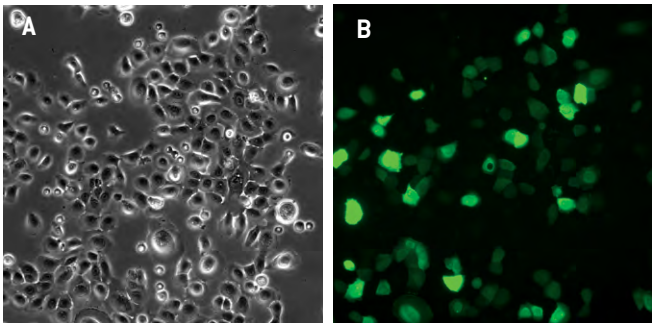
BioResearch

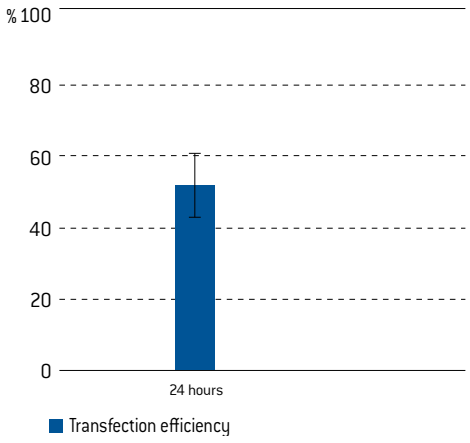
# Amaxa™ Nucleofector™ Protocol for Normal Human Bronchial Epithelial Cells (NHBE)

Validated to work with Clonetics™ NHBE [Lonza; Cat. No. CC-2540]; adherent epithelial cells




**Example for Nucleofection of NHBE Cells**

NHBE cells were transfected using program W-001 and a plasmid encoding the maxGFP™ fluorescent protein. 24 hours post Nucleofection the cells were analyzed by light (A) and fluorescence microscopy (B).




**Average transfection efficiency of NHBE cells 24 hours post Nucleofection.** Cells were transfected using Nucleofector™ Program W-001 and 2 µg of pmaxGFP™ Vector. Cell viability is usually around 50%.

## Product Description

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

| Cat No. | VPI-1005 |
|---|---|
| Size [reaction] | 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 Cuvette | 25 |
| Pastic Pipetts | 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

### Note

4D-Nucleofector™ Solutions can only be used with conductive polymer Nucleocuvette™ Vessels, i.e. in the 4D-Nucleofector™ and the 96-well Shuttle™ System. They are not compatible with the Nucleofector™ II/2b Device.

## 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:** ReagentPack™ Subculture Reagent Kit containing trypsin/EDTA, HEPES Buffered Saline Solution (HBSS) and Trypsin Neutralizing Solution (TNS) [Lonza, Cat. No. CC-5034]
- **Culture medium:** BEGM™ BulletKit™ [Lonza; Cat. No. CC-3170])
- Prewarm appropriate volume of culture media at 37°C (1.5 mL per reaction)
- Appropriate number of cells (4–5 x $10^5$ cells per sample; minimal cell number: 4 x $10^5$ cells; a lower cell number may lead to a major increase in cell mortality

## 1. Pre Nucleofection

### Cell culture recommendations

1.1 Seeding conditions: at least 3.5 x $10^3$ cells/$cm^2$
1.2 Replace media 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 Cells are very sensitive to trypsin treatment. For harvesting, incubate the cells 5 – 6 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material); if cells are incubated >7 – 10 minutes cells may start to clump
1.9 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 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 1.9–1.11)
2.4 Count an aliquot of the trypsinized cells and determine cell density
2.5 Centrifuge the required number of cells (5 x 105 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 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 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 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

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