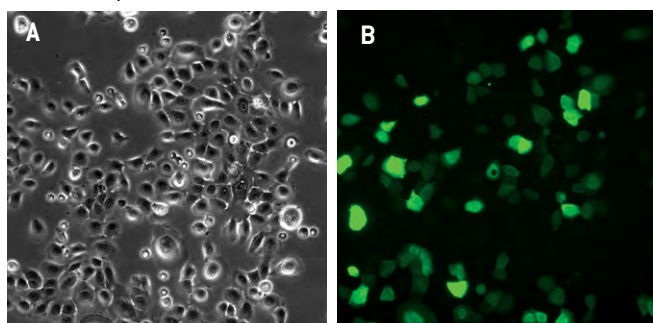


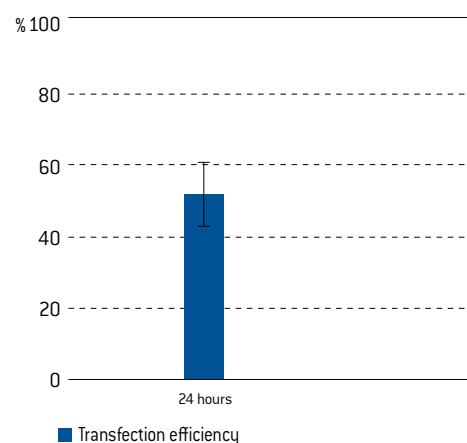
# Amaxa™ Nucleofector™ Protocol for Normal Human Bronchial Epithelial Cells (NHBE)

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<sup>5</sup> cells per sample; minimal cell number: 4 x 10<sup>5</sup> 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<sup>3</sup> cells/cm<sup>2</sup>
- 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 <sup>5</sup> cells
1 – 5 µg plasmid DNA (in 1 – 5 µL H <sub>2</sub> O 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<sub>2</sub> 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 10<sup>5</sup> 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<sub>2</sub> 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](http://www.lonza.com/nucleofection-citations)

For more technical assistance, contact our Scientific Support Team:

### USA /Canada

Phone: 800 521 0390 (toll-free)  
Fax: 301 845 8338  
E-mail: [scientific.support@lonza.com](mailto:scientific.support@lonza.com)

### Europe and Rest of World

Phone: +49 221 99199 400  
Fax: +49 221 99199 499  
E-mail: [scientific.support.eu@lonza.com](mailto:scientific.support.eu@lonza.com)

---

**Lonza Cologne GmbH**  
50829 Cologne, Germany

Please note that the Nucleofector™ Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

The Nucleofector™ Technology, comprising Nucleofection Process, Nucleofector™ Device, Nucleofector™ Solutions, Nucleofector™ 96-well Shuttle™ System and 96-well Nucleocuvette™ plates and modules is covered by patent and/or patent-pending rights owned by Lonza Cologne GmbH.

Nucleofector, Nucleofection, 4D-Nucleofector, Nucleocuvette and maxGFP are registered trademarks of the Lonza Cologne GmbH in Germany and/or U.S. and/or other countries.

Other product and company names mentioned herein are the trademarks of their respective owners.

This kit contains a proprietary nucleic acid coding for a proprietary copepod fluorescent protein intended to be used as a positive control with this Lonza product only. Any use of the proprietary nucleic acid or protein other than as a positive control with this Lonza product is strictly prohibited. USE IN ANY OTHER APPLICATION REQUIRES A LICENSE FROM EVROGEN. To obtain such a license, please contact Evrogen at [license@evrogen.com](mailto:license@evrogen.com).

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

All trademarks belong to Lonza or its affiliates or to their respective third party owners. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group Ltd or its affiliates and the information and recommendations given by Lonza Group Ltd or its affiliates are (i) suitable for intended process or purpose, (ii) in compliance with environmental, health and safety regulations, and (iii) will not infringe any third party's intellectual property rights.

© 2017 Lonza. All rights reserved.  
DPK-1001\_2017-08

---