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Amaxa™ Basic Nucleofector™ Kit for Primary Mammalian Epithelial Cells

For Primary Mammalian Epithelial Cells

Cells derived from mammalian epithelial tissues from various organs; adherent cells

Note **Mammalian epithelial cells display significant phenotypic variations due to the wide range of both species and body sites from which they may be sourced. You can determine the optimal Nucleofection™ condition for your epithelial cells using the Basic Nucleofector™ Kit for Primary Mammalian Epithelial Cells [Cat.No. VPI-1005]. Please find some guidelines on epithelial cell culture for Nucleofection™ and on the transfection procedure using our Basic Nucleofector™ Kit below. However, we recommend referring to more detailed culture protocols before you start the experiments. Having tested various epithelial cell types, high transfection efficiencies could be achieved using one of the programs indicated below. If you do not attain satisfying results with your epithelial cells of interest, please contact our Scientific Support Team for further help with the optimization. On our website (www.lonzabio.com) we provide a form you might use to enter the results achieved with the Basic Kit.**

Product Description

Cat. No.	**VPI-1005**
Size (Reactions)	25
Basic Nucleofector™ Solution for Mammalian Epithelial Cells	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.

Optimization Guidelines

The initial optimization experiment is comprised of 6 reactions: 5 different Nucleofector™ Programs are tested with 1 Nucleofector™ Solution plus 1 control (no program). The Nucleofector™ Program which turns out to be the most appropriate should be used for all subsequent transfections. A further fine tuning of the Nucleofection™ condition can be performed with the help of our Scientific Support Team.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Program	S-005	T-013	T-020	W-001	U-017	No program

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
- Culture dishes (Ø 6 cm) 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:** Please use a medium especially suited for the culture of primary epithelial cells, e.g. Epithelial Cell Medium, MEGM® Basal Medium [Lonza, Cat. No. CC-3151] with BulletKit® Additives [Lonza, Cat. No. CC-3150] and SingleQuot [Lonza, Cat. No. CC-4136] or a different special medium recommended for your epithelial cell type containing all required supplements. **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 medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (0.5 – 1.5 10^6 cells per sample)
  Minimal cell number: 2 x 10^5 cells (a lower cell number may lead to major in crease in cell mortality)
  Maximum cell number: 5 x 10^6

1. Pre Nucleofection™

Note **Transfection results may be donor-dependent.**
Culture conditions may differ between cell types. Please follow your established procedure or the supplier's recommendations.

Cell culture recommendations

1.1 Seeding conditions: 2 – 6 x 10^3 cells/cm²
1.2 Replace medium every 2 days (6 – 7 ml per 75 cm² flask) at about 75% confluency
1.3 Cells should be passaged after reaching 80% confluency; higher confluency may reduce viability of the cells
1.4 Do not use cells after passage 9 for Nucleofection™
1.5 Cells should be passaged 3 – 5 days before Nucleofection™

Trypsinization

1.6 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media

1.7 For harvesting, incubate the cells 4 – 6 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.8 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 Nucleofector™ Sample contains
0.5 – 1.5 x 10^6 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 culture dishes (Ø 6 cm) by filling appropriate number of wells with 3 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 1.6 – 1.8)
2.4 Count an aliquot of the trypsinized cells and determine cell density
2.5 Centrifuge the required number of cells (0.5 – 1.5 x 10^6 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 (recommended for initial optimization) 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. Please try all 5 Nucleofector™ Programs initially to determine the most appropriate one for your epithelial cell type for all subsequent experiments **S-005, T-013, T-020, W-001, U-017** (S-05, T-13, T-20, W-01 and U-17 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 culture dish (Ø 6 cm) (final volume 3 ml media per well). 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 but ideally, cells should be left undisturbed for 24 hours

Additional Information

For an up-to-date list of all primary mammalian epithelial cells successfully transfected with this Basic Nucleofector™ Kit, please refer to:
www.lonza.com/cell-database

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:

USA /Canada
Phone: 800 521 0390 (toll-free)
Fax: 301 845 8338
E-mail: 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

Lonza Cologne GmbH
50829 Cologne, Germany

Please note that the Amaxa™ 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.

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