BioResearch

Lonza

Amaxa™ 96-well Shuttle™ Basic Protocol for Primary Mammalian Epithelial Cells

Cell Description

Epithelial cells, adherent.

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.

This basic protocol describes how to easily define optimal Nucleofection™ Conditions for different mammalian epithelial cells. We recommend to first test a set of pre-selected Nucleofector™ Programs together with two of our Primary Cell 96-well Nucleofector™ Kits:

- P1 Primary Cell 96-well Nucleofector™ Kit
- P3 Primary Cell 96-well Nucleofector™ Kit

For subsequent experiments simply use the kit which yields the best results.

If you have questions regarding your epithelial cells of interest, please contact our Scientific Support Team for further help with the optimization.

Product Description

Recommended Kits

P1 Primary Cell 96-well Nucleofector™ Kit or
P3 Primary Cell 96-well Nucleofector™ Kit

Cat. No.	V4SP-1096
Size (reactions)	1×96
P1 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	1

Cat. No.	V4SP-1960
Size (reactions)	10×96
P1 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5.0 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	10

Cat. No.	V4SP-3096
Size (reactions)	1×96
P3 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	1

Cat. No.	V4SP-3960
Size (reactions)	10×96
P3 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	10

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 expiry 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

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

Optimization Guidelines

The initial optimization experiment is comprised of 64 reactions, using 4 Nucleocuvette™ Modules: 7 different Nucleofector™ Programs are tested in duplicate with 2 Nucleofector™ Solutions plus 1 control with 2 different cell numbers (0.5×10^5 and 1×10^5). The program and 96-well Nucleofector™ Solution which turns out to be the most appropriate Nucleofection™ Condition 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.

	P1 Primary Cell Nucleofector™ Solution				P3 Primary Cell Nucleofector™ Solution				
	0.5×10^5 cells/sample		1×10^5 cells/sample		0.5×10^5 cells/sample		1×10^5 cells/sample		
	1	2	3	4	5	6	7	8	9–12
A	96-CM-102	96-CM-102	96-CM-102	96-CM-102	96-CM-102	96-CM-102	96-CM-102	96-CM-102	—
B	96-DC-100	96-DC-100	96-DC-100	96-DC-100	96-DC-100	96-DC-100	96-DC-100	96-DC-100	—
C	96-EA-104	96-EA-104	96-EA-104	96-EA-104	96-EA-104	96-EA-104	96-EA-104	96-EA-104	—
D	96-EL-110	96-EL-110	96-EL-110	96-EL-110	96-EL-110	96-EL-110	96-EL-110	96-EL-110	—
E	96-ED-100	96-ED-100	96-ED-100	96-ED-100	96-ED-100	96-ED-100	96-ED-100	96-ED-100	—
F	96-CM-113	96-CM-113	96-CM-113	96-CM-113	96-CM-113	96-CM-113	96-CM-113	96-CM-113	—
G	96-DS-109	96-DS-109	96-DS-109	96-DS-109	96-DS-109	96-DS-109	96-DS-109	96-DS-109	—
H	negative control (no program)	negative control (no program)	negative control (no program)	negative control (no program)	negative control (no program)	negative control (no program)	negative control (no program)	negative control (no program)	—

The P1 and P3 Primary Cell 96-well Nucleofector™ Kits have been tested successfully for the following Clonetics™ Primary Cells provided by Lonza:

Cell type	Lonza Cat. No.	Optimal Program	Optimal Solution	Transfection efficiency	Viability
HMEC (Human Mammary Epithelial Cells)	CC-2551	96-EL-110	P3	51 %	66 %
NHBE (Normal Human Bronchial Epithelial Cells)	CC-2540 / CC-2541	96-DC-100	P3	53 %	54 %
PrEC (Human Prostate Epithelial Cells)	CC-2555	96-CM-102	P1	67 %	48 %
SAEC (Human Small Airway Epithelial Cells)	CC-2547	96-EL-110	P3	64 %	75–100 %

Required Material

Note
Please make sure that the entire supplement is added to the Nucleofector™ Solution.

- Nucleofector™ 96-well Shuttle System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented Nucleofector™ Solutions at room temperature
- Supplied Nucleocuvette™ Plate(s)
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µl

Note
Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin free kits; A260 : A280 ratio should be at least 1.8
- Nucleocuvette™ compatible tips: epT.I.P.S.™ (US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266), Matrix TallTips™ (Matrix Technologies Corp., Cat. No. 7281) or LTS Tips (Rainin Instrument, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S). Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- 96-well culture plates or culture plates of your choice
- For trypsinization: Please use trypsin as recommended by the cells supplier e.g. 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 media as recommended by the cell supplier, e.g. Lonza (PrEGM™ BulletKit™ for PrEC (Cat. No. CC-3166), MEGM™ BulletKit™ for HMEC (Cat. No. CC-3150), SAGM™ BulletKit™ for SAEC (Cat. No. CC-3118) or BEGM™ BulletKit™ for NHBE (Cat. No. CC-3170)) or the established standard growth media for the epithelial cells your are using supplemented with a suitable antimicrobial and e.g. 10 % FCS
- Prewarm appropriate volume of culture media at 37°C (230 µl per sample)
- Appropriate number of cells (0.5–1×10^5 cells per sample)

1. Pre Nucleofection™

Note
Transfection results may be source-dependent.

Cell Culture Recommendations

1.1 Seeding conditions: 2–6×10^3 cells/cm^2
1.2 Replace medium 2–3 times a week (15 ml per 75 cm^2 flask)
1.3 Cells should be passaged after reaching 80 % confluency
1.4 Do not use cells after passage 9 for Nucleofection™
1.5 Cells should be passaged 2 days before Nucleofection™ depending on growth rate of cells

Note
Culture conditions may differ between cell types. Please follow your established procedure or the supplier's recommendations.

Trypsinization

Note
Please follow your established procedure or the supplier's recommendations (e.g. for Reagent Pack™ Subculture Reagent Kit follow procedure described below).

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 Nucleofection™ Sample Contains

- 0.5–1×10^5 cells
- 0.4–1 µg plasmid DNA (in 1–2 µl H_2O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample) recommended for initial optimization
- 20 µl P1 or P3 Primary Cell 96-well Nucleofector™ Solutions

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution

2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see Manual "Nucleofector™ 96-well Shuttle™ System")

2.3 Select appropriate 96-well Shuttle™ Program. Please try all 7 96-well Shuttle™ Programs (**96-CM-102, 96-CM-113, 96-DC-100, 96-EA-104, 96-EL-110, 96-ED-100 and 96-DS-109**) initially with both 96-well Nucleofector™ Solutions to determine the most appropriate 96-well Nucleofection™ Condition for your specific epithelial cell type

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 150 µl* (see note at the end of this chapter) for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

2.5 Pre-warm an aliquot of culture media to 37°C (80 µl* per sample)

2.6 Prepare 0.4–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector (recommended for initial optimization). For siRNA experiments we recommend to start using 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Harvest the cells by trypsinization (please see 1.6–1.8)

2.8 Count an aliquot of the trypsinized cells and determine cell density

2.9 Centrifuge the required number of cells (0.5×10^5 or 1×10^5 cells per sample) at 220×g for 5 minutes at room temperature

2.10 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B: Multiple substrates (e.g. Library Transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability it is important to work as quickly as possible. Avoid air bubbles while pipetting.

2.11 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well

2.12 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well "A1" must be in upper left position

2.13 Start 96-well Nucleofection™ Process by either pressing "Upload and start" in the 96-well Shuttle™ Software or pressing "Upload" in the 96-well Shuttle™ Software and then the "Start" button at the 96-well Shuttle™ (for both options please refer to the respective Manual)

2.14 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer

2.15 Incubate Nucleocuvette™ Plate 10 minutes at room temperature. Please note that this incubation may reduce cell viability and should be avoided if viability is a concern

2.16 Resuspend cells with desired volume of pre-warmed media (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 80 µl* of pre-warmed media

2.17 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 50 µl of resuspended cells to 150 µl pre-warmed media prepared in 96-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ Results in most cases, however, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

3. Post Nucleofection™

3.1 Incubate the cells in humidified 37°C/5 % CO_2 incubator until analysis. Gene expression is often detectable after only 4–8 hours

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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