BioResearch

Lonza

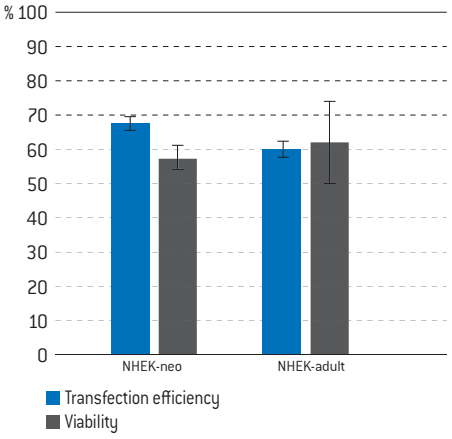
Amaxa™ 4D-Nucleofector™ Protocol for Normal Human Epidermal Keratinocytes (NHEK)

For 4D-Nucleofector™ X Unit (Transfection in suspension)

Validated to work with neonatal and adult Clonetics™ KGM-Gold™ Keratinocytes (Lonza; Cat. No. 00192907 (NHEK-Neo); Cat. No. 00192906 (NHEK-Neo, pooled); Cat. No. 00192627 (NHEK-adult)]; adherent epithelial cells

Example for Nucleofection™ of NHEK Cells

Transfection efficiency of NHEK-neo/NHEK-adult cells 24 hours post Nucleofection™. 1.0 x 10^5 cells were transfected with program DS-138 using 0.4 µg pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. Cells were analyzed 24 hours post Nucleofection™ using a FACSCalibur™ (Becton Dickinson). Cell viability was measured with the ViaLight™ Plus Bioassay Kit; Lonza, Cat. No. LT07-221).



Product Description

Recommended Kit(s)–P3 Primary Cell 4D-Nucleofector™ X Kit

Cat No.	V4XP-3012	V4XP-3024	V4XP-3032
Transfection volume	100 µl	100 µl	20 µl
Size (reaction)	2 x 6	24	2 x 16
Nucleofector™ Solution	2 x 0.675 ml (0.492 ml + 27% overfill)	2.25 ml (1.968 ml + 13% overfill)	0.675 ml (0.525 ml + 22% overfill)
Supplement	2 x 0.15 ml (0.108 ml + 27% overfill)	0.5 ml (0.432 ml + 13% overfill)	0.15 ml (0.115 ml + 22% overfill)
pmaxGFP™ Vector (1.0 µg/µl in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg
Single Nucleocuevette™ (100 µl)	12	24	-
16-well Nucleocuvette™ Strips (20 µl)	-	-	2

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 could be only used with conductive polymer Nucleovettes™, 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 (see table 1)

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 100 µl single Nucleocuvette™ or 20 µl 16-well Nucleocuvette™ Strips
- Compatible tips for 20 µl Nucleocuvette™ Strips: 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
- 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
- Cell culture plates of your choice
- For detaching cells: 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**: KGM-Gold™ BulletKit™ [Lonza; Cat. No. 00192060] Prewarm appropriate volume of culture medium to 37°C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection™

Note
Transfection results may be donor-dependent.

Cell culture recommendations

Neonatal NHEK

1.1 Seeding conditions: 5–7 x 10^3 cells/cm^2; use e.g. 75cm^2 flasks
1.2 Cells should be passaged after reaching 60–70% confluency; replace media every other day
1.3 For Nucleofection™ cells should be preferably passaged 3–4 days before; optimal confluency before Nucleofection™ is 80–90%
1.4 Do not use cells after passage number 5 as this may result in substantially lower gene transfer efficiency and viability

Adult NHEK

1.5 Seeding conditions: 5 x 10^3 cells/cm^2; use e.g. 75cm^2 flasks
1.6 Cells should be passaged after reaching 50–60% confluency; replace media every other day
1.7 For Nucleofection™ cells should be preferably passaged 3 days before; optimal confluency before Nucleofection is 50–60%
1.8 Do not use cells after passage number 2 as this may result in substantially lower gene transfer efficiency and viability

Trypsinization

1.9 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
1.10 Cells are very sensitive to trypsin treatment. For harvesting, incubate the cells 3–5 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.11 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached. Do not incubate the cells in TNS longer than 10 minutes

2. Nucleofection™

For Nucleofection™ Sample contents and recommended Nucleofector™ Program, please refer to Table 3.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)
2.3 Select/Check for the appropriate Nucleofector™ Program (see table 3)
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media (see table 4) and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.5 Pre-warm an aliquot of culture medium to 37°C (see table 4)
2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)
2.7 Harvest the cells by trypsinization (please see 1.9–1.11)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (see table 3) at 200xg for 10 minutes at room temperature. Remove supernatant completely
2.10 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see table 3)
2.11 Prepare mastermixes by dividing cell suspension according to number of substrates

2.12 Add required amount of substrates to each aliquot (max. 10 % of final sample volume)
2.13 Transfer mastermixes into the Nucleocuvette™ Vessels

Note
As leaving cells in 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.

2.14 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
2.15 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.16 Start Nucleofection™ Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.17 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.18 Incubate Nucleocuvette™ for 10 min at room temperature
2.19 Resuspend cells with pre-warmed medium (for recommended volumes see table 5). Mix cells by gently pipetting up and down two to three times. When working with the 100 µl Nucleocuvette™ use the supplied pipettes and avoid repeated aspiration of the sample
2.20 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

3. Post Nucleofection™

3.1 Incubate the cells in 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

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

D4XP-3016_2011-01

Table 1: Volumes required for a single reaction

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Volume of Nucleofector™ Solution	82 µl	16.4 µl
Volume of Supplement	18 µl	3.6 µl

Table 2: Required amounts of cells and media for Nucleofection™

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Culture medium per sample post Nucleofection™ (for transfer and culture)	2 ml	150 µl
Cell number per Nucleofection™ Sample	5 x 10^5 cells; minimal cell number: 4 x 10^5 cells; a lower cell number may lead to a major increase in cell mortality	1 x10^5 cells (Lower or higher cell numbers may influence transfection results)

Table 3: Contents of one Nucleofection™ Sample and recommended program

		100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells		5 x 10^5	1 x 10^5
Substrate*	**pmaxGFP™ Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	1–5 µg	0.4–1 µg
or	**siRNA**	30–300nM siRNA (3–30 pmol/sample)	30–300nM siRNA (0.6–6 pmol/sample)
P3 Primary Cell 4D-Nucleofector™ X Solution		100 µl	20 µl
Program		DS-138	DS-138

* Volume of substrate should comprise maximum 10 % of total reaction volume

Table 4: Culture volumes required for post Nucleofection™ Steps

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
12-well culture plate	1.5 ml	-
96-well culture plate	-	70 µl
Culture medium to be added to the sample post Nucleofection™	500 µl	80 µl

* Maximum cuvette volume 200 µl

Table 5: Recommended volumes for sample transfer into culture plate

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	500 µl	80 µl
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	30 µl

* Maximum cuvette volume 200 µl