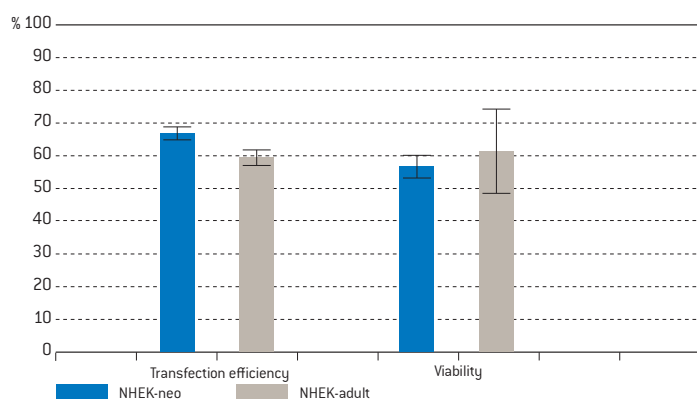


# Amaxa™ HT Nucleofector™ protocol for normal human epidermal keratinocytes (NHEK)

## Cell description

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 efficiencies of NHEK-neo/NHEK-adult cells 24 hours post Nucleofection™.**  $1.0 \times 10^5$  cells were transfected with program DS-138-AA using  $0.4 \mu\text{g}$  pmaxGFP™ vector. 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 HT Nucleofector™ kits

|  |                  |
|--|------------------|
| Cat. No.   | V5SP-3002        |
| Size (reactions)   | 2×384            |
| P3 primary cell HT Nucleofector™ solution                            | 22.5 ml          |
| Supplement   | 5 ml             |
| pmaxGFP™ vector (1.0 $\mu\text{g}/\mu\text{l}$ in 10 mM Tris pH 8.0) | 50 $\mu\text{g}$ |
| 384-well Nucleocuvette™ plate(s)                                     | 2                |

|  |                   |
|--|-------------------|
| Cat. No.   | V5SP-3010         |
| Size (reactions)   | 10×384            |
| P3 primary cell HT Nucleofector™ solution                            | 90 ml             |
| Supplement   | 20 ml             |
| pmaxGFP™ vector (1.0 $\mu\text{g}/\mu\text{l}$ in 10 mM Tris pH 8.0) | 150 $\mu\text{g}$ |
| 384-well 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 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

HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, the Shuttle™ device and in the 4D-Nucleofector™ 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.

- HT Nucleofector™ system
- Supplemented HT Nucleofector™ solution at room temperature
- Supplied 384-well Nucleocuvette™ plate(s)
- Supplied pmaxGFP™ vector, stock solution 1 µg/µl
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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

### 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
- 96-well culture plates or culture plates of your choice
- Culture medium: KGM-Gold™ BulletKit™ [Lonza; Cat. No. 00192060]
- Pre-warm appropriate volume of culture medium to 37°C (122 µl per sample)
- 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]
- Appropriate number of cells (1 x 10<sup>5</sup> cells per sample; lower or higher cell numbers may influence transfection results)

## 1. Pre Nucleofection™

### Note

Transfection results may be donor-dependent.

### Cell culture recommendations

#### Neonatal NHEK

- 1.1 Seeding conditions: 5–7 x 10<sup>3</sup> cells/cm<sup>2</sup>; use e.g. 75cm<sup>2</sup> flasks
- 1.2 Cells should be passaged after reaching 60–70% confluency; replace media every other day

- 1.3 Cells should be preferably passaged 3–4 days before Nucleofection™; 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<sup>3</sup> cells/cm<sup>2</sup>; use e.g. 75cm<sup>2</sup> flasks
- 1.6 Cells should be passaged after reaching 50–60% confluency; replace media every other day
- 1.7 Cells should be preferably passaged 3 days before Nucleofection™; 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™

### One Nucleofection™ sample contains

- 1 x 10<sup>5</sup> cells
  - 0.4–1 µg plasmid DNA (in 1–2 µl H<sub>2</sub>O or TE) or 0.4 µg pmaxGFP™ vector or 30–300nM siRNA (0.6–6 pmol/sample)
  - 20 µl P3 primary cell HT Nucleofector™ solution
- 2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution
  - 2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details refer to the HT Nucleofector™ manuals)
  - 2.3 Select the appropriate HT Nucleofector™ program **DS-138-AA**
  - 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 82 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<sub>2</sub> incubator
  - 2.5 Pre-warm an aliquot of culture medium to 37°C (40 µl per sample)
  - 2.6 Prepare 0.4–1 µg plasmid DNA or 0.4 µg pmaxGFP™ vector or 30 nM–300 nM siRNA (0.6–6 pmol/sample)

- 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 ( $1 \times 10^5$  cells per sample) at 200xg for 10 minutes at room temperature. Remove supernatant completely
- 2.10 Resuspend the cell pellet carefully in 20  $\mu$ l room temperature HT 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  $\mu$ l per sample)
- Transfer 20  $\mu$ l of mastermixes into the wells of the 384-well Nucleocuvette™ plates

#### B. Multiple substrates (e.g. library transfection):

- Pipette 20  $\mu$ l of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
- Add 2  $\mu$ l substrates (maximum) to each well
- Transfer 20  $\mu$ l of cells with substrates into the wells of the 384-well Nucleocuvette™ plates

#### Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a liquid handling system or at least a multi-channel pipette with suitable pipette tips. As leaving cells in HT 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 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate
- 2.12 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well “A1” must be in upper left position
- 2.13 Start Nucleofection™ process clicking “Start” in the HT Nucleofector™ software (for details refer to the HT Nucleofector™ manuals)
- 2.14 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel
- 2.15 Resuspend cells with desired volume of pre-warmed culture medium (maximum cuvette volume 60  $\mu$ l). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40  $\mu$ l of pre-warmed media\*
- 2.16 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 18  $\mu$ l of resuspended cells to 82  $\mu$ l pre-warmed media

#### \* Note

The indicated cell numbers and volumes have been found to produce optimal 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<sub>2</sub> incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours

## Additional information

### Up-to-date list of all Nucleofector™ references

[www.lonza.com/nucleofection-citations](http://www.lonza.com/nucleofection-citations)

### Technical assistance and scientific support

#### USA/Canada

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Fax 301 845 8338

[scientific.support@lonza.com](mailto:scientific.support@lonza.com)

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Fax +49 221 99199 499

[scientific.support.eu@lonza.com](mailto:scientific.support.eu@lonza.com)

## [www.lonza.com](http://www.lonza.com)

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