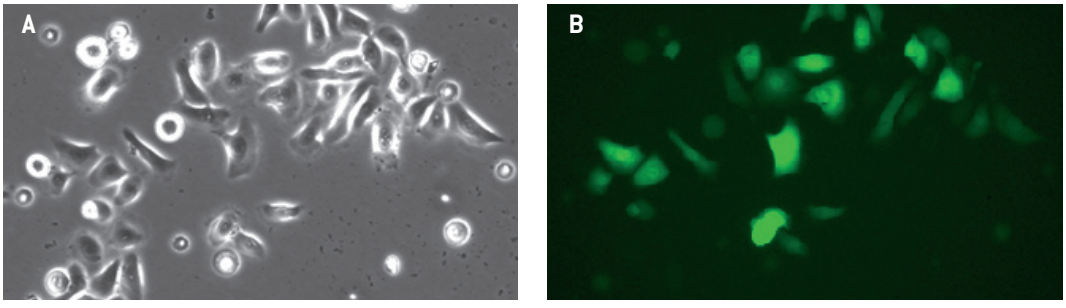
Amaxa® Human Keratinocyte Nucleofector® Kit

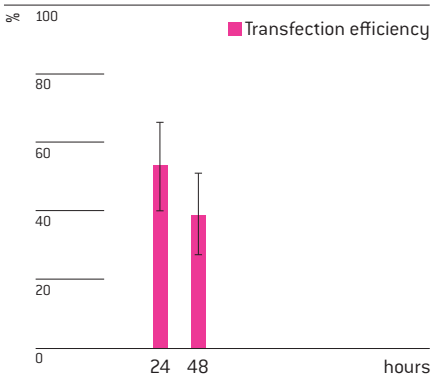
For Human Keratinocytes - Neonatal

Human Keratinocytes - Neonatal [Invitrogen; Cat. No. C-001-5C]; isolated form neonatal foreskin; undifferentiated adherent epidermal cells with cobble stone-like morphology which changes upon differentiation

Example for Nucleofection® of primary neonatal human keratinocytes



Primary human neonatal keratinocytes were transfected using the Human Keratinocyte Nucleofector® Kit, program T-007 and 2.5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. 24 hours post Nucleofection®, the cells were analyzed by light (A) and fluorescence microscopy (B).



Transfection efficiencies of human neonatal keratinocytes 24 and 48 hours post Nucleofection®. Cells from various lots were transfected with program T-018 and a plasmid encoding the enhanced green fluorescent protein eGFP. Cells were analyzed by flow cytometry and cell viability usually varies between 50 – 60%.

Product Description

Cat. No.	**VPD-1002**
Size (Reactions)	25
Human Keratinocyte 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 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.

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**: Trypsin/EDTA Solution [Invitrogen, Cat. No. R-001-100] and Trypsin Neutralizing Solution (TNS) [Invitrogen, Cat. No. R-002-100]
- **Culture medium:** EpiLife® Medium [Invitrogen, Cat. No. M-EPI-500] supplemented with Human Keratinocyte Growth Supplement HKGS [Invitrogen, Cat. No. S-001-5] or Keratinocytes - SFM [Invitrogen, Cat. No. 17005-042] mixed 1 : 1 with MCDB 153 medium [Biochrom, Cat. No. F8115]
- Prewarm appropriate volume of culture media at 37°C (1.5 ml per sample)
- Appropriate number of cells (0.5 – 1 x 10^6 cells per sample)
  Minimal cell number: 3 x 10^5 (a lower cell number may lead to major increase in cell mortality)
  Maximum cell number: 1 x 10^6

1. Pre Nucleofection®

Note **Transfection results may be donor - dependent.**

Cell culture recommendations

1.1 Seeding conditions: 2.5 x 10^3 cells/cm^2
1.2 Replace media every 2 – 3 times per week; 2 – 3 ml per 25 cm^2 flask
1.3 Cells should be passaged after reaching 60 – 70% confluency
1.4 For Nucleofection® cells should be preferably passaged 4 – 5 days before
1.5 Do not use cells after passage number 5 as this may result in substantially lower gene transfer efficiency and viability. Also cell detachment using trypsin treatment becomes more difficult and may damage the cells
1.6 Optimal confluency before Nucleofection® 60 – 70%. Higher confluency may reduce viability

Trypsinization

1.7 Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture media
1.8 For harvesting, incubate the cells 5 – 7 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.9 Neutralize trypsinization reaction with Trypsin Neutralizing Solution once the majority of the cells (>90%) have been detached

2. Nucleofection®

One Nucleofection® Sample contains
0.5 – 1 x 10^6 cells
2 – 3 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 Human Keratinocyte Nucleofector® Solution

2.1 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_2 incubator
2.2 Harvest the cells by trypsinization (please see 1.7 – 1.9)
2.3 Count an aliquot of the trypsinized cells and determine cell density
2.4 Centrifuge the required number of cells (**0.5 x 106 cells per sample**) **at 200xg for 7 minutes** at room temperature
2.5 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample
2.6 Combine 100 µl of cell suspension with **2 – 3 µg DNA**, 2 µg pmaxGFP® Vector or **30 nM – 300 nM** siRNA (3 – 30 pmol/sample) or other substrates
2.7 Transfer cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles. Close the cuvette with the cap
2.8 Select the appropriate Nucleofector® Program **T-007** for high viability or **T-018** for high transfection efficiency (T-07 or T-18 for Nucleofector® I Device)
2.9 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.10 Take the cuvette out of the holder once the program is finished
2.11 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_2 incubator until analysis and change medium 24 hours post Nucleofection®
3.2 Gene expression or down regulation, respectively, is often detectable after only 4 – 8 hours but ideally, cells should be left undisturbed until medium change 24 hours post Nucleofection®, as cells may, just like freshly thawed cells, need longer to attach

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:

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