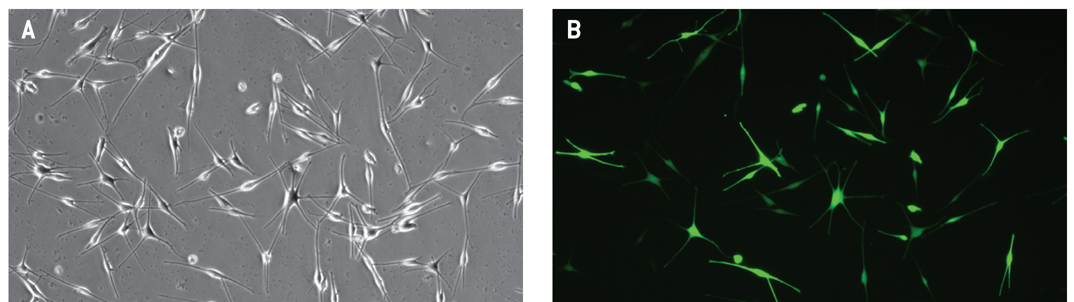
Amaxa® NHEM-Neo Nucleofector® Kit

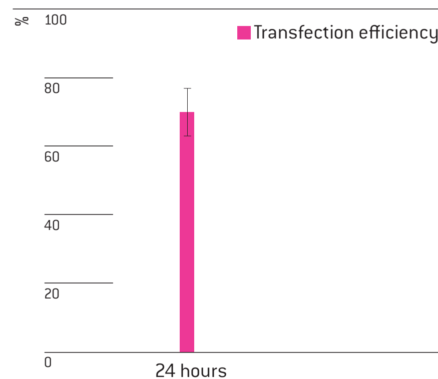
For Normal Human Epidermal Melanocytes, Neonatal (NHEM-neo)

NHEM-Neo [Invitrogen, Cat. No. C-002-5C]; small adherent cells with long protrusions and a star or spindle-like form

Example for Nucleofection® of Human NHEM-Neo with eGFP cDNA



Primary human NHEM-Neo were transfected using the Human NHEM Nucleofector® Kit, program U-024 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).



Average transfection efficiency of human NHEM-Neo. Primary human NHEM-Neo were transfected with Nucleofector® Program U-024 and 5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. 24 hours post Nucleofection®, the cells were analyzed by flow cytometry. Cell viability is around 55 – 60%.

Product Description

Cat. No.	**VPD-1003**
Size (Reactions)	25
NHEM-Neo 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 [Invitrogen, Cat. No. R-001-100] and Trypsin Neutralizer Solution [Invitrogen, Cat. No. R-002-100]
- **Culture medium**: Medium 254CF [Invitrogen, Cat. No. M-254CF-500] supplemented with Human Melanocyte Growth Supplement HMGS [Invitrogen, Cat. No. S-002-5] and PSA solution
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (5 x 10^5 cells per sample)
  Minimal cell number: 2 x 10^5 cells (a lower cell number may lead to a 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: 1 x 10^4 cells/cm²
1.2 Replace media every 2 days; 6 – 7 ml per 75 cm² flask
1.3 Cells should be passaged after reaching 80 – 90% confluency
1.4 For Nucleofection® cells should be preferably passaged 5 – 7 days before
1.5 Do not use cells after passage number 10 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®: 80%. 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 Neutralizer Solution once the majority of the cells (>90%) have been detached

2. Nucleofection®

One Nucleofection® Sample contains
5 x 10^5 cells
1 – 2 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 NHEM-Neo 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 (**5 x 10^5 cells per sample**) **at 90xg 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 **1 – 2 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 **U-024** or **U-016** for self isolated cells (**U-24** or **U-16** 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. 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 AG
50829 Cologne, Germany

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