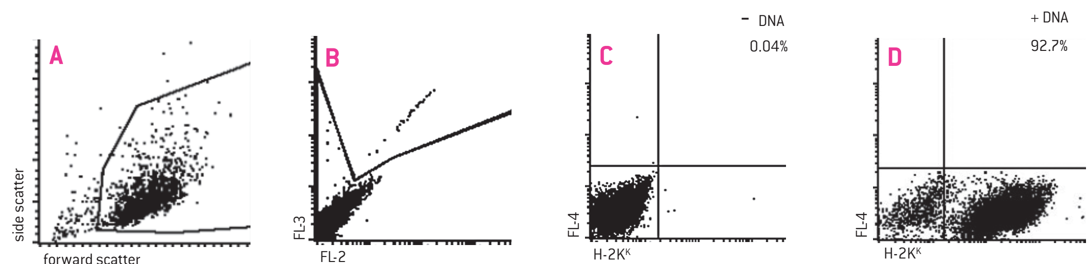
Lonza

Amaxa® Human Dermal Fibroblasts Nucleofector® Kit

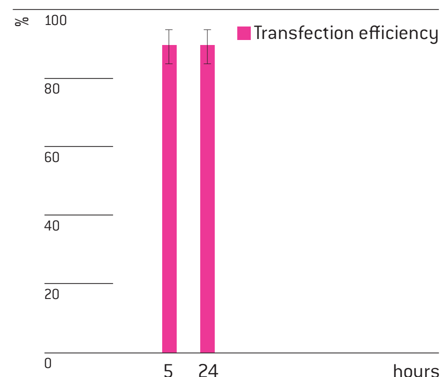
For Normal Human Dermal Fibroblasts – Neonatal (NHDF-Neo)

Validated to work with Clonetics® NHDF-Neo [Lonza; Cat. No. CC-2509]; adherent fibroblastoid cells

Example for Nucleofection® of NHDF-Neo



NHDF-Neo were transfected using the Human Dermal Fibroblast Nucleofector® Kit, program U-020 and a plasmid encoding the mouse MHC class I heavy chain molecule, H-2K^k. 5 hours post Nucleofection® the cells were stained with a Cy5-coupled antibody directed against H-2K^k and were analyzed by flow cytometry. NHDF-Neo were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). H-2K^k expression of NHDF-Neo is shown after Nucleofection® without (C) and with plasmid DNA (D).



Transfection efficiencies of NHDF-Neonatal 5 and 24 hours post Nucleofection®. Cells were transfected using program U-020 and a plasmid encoding the mouse MHC class I heavy chain molecule H-2K^k. Viability is usually around 85 – 90%.

Product Description

Cat. No.	**VPD-1001**
Size (Reactions)	25
Human Dermal Fibroblast 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
- **For trypsinization**: Reagent Pack™ Subculture Reagent Kit containing Trypsin/EDTA, HEPES Buffered Saline Solution (HBSS) and Trypsin Neutralizing Solution (TNS) [Lonza, Cat. No. CC-5034]. Alternatively if cells hardly detach: Trypsin 0.5 % – EDTA 0.2%
- **Culture medium:** FGM®-2 BulletKit [Lonza; Cat. No. CC-3132]. **We recommend storing 40 ml aliquots of the prepared medium at -80°C. Do not use medium stored at 4°C for more than two or three days, as this may lead to reduced cell viability**
- Prewarm appropriate volume of culture medium to 37°C (1.5 ml per sample)
- Appropriate number of cells (0.5 – 1 x 10^6 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: 2 x 10^6

1. Pre Nucleofection®

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

Cell culture recommendations

1.1 Seeding conditions: at least 5 x 10^4 cells/25cm² flask
1.2 Replace media 2 – 3 times per week; 2 – 3 ml media per 25 cm² flask
1.3 Cells should be passaged after reaching 90% confluency
1.4 Cells should be preferably passaged 2 days before Nucleofection®
1.5 Do not use cells after passage number 15 as this may result in substantially lower gene transfer efficiency and viability
1.6 Optimal confluency before Nucleofection® 90%

Trypsinization

1.7 Remove media from the cultured cells and wash cells once with HBSS; use at least same volume of HBSS as culture media
1.8 For harvesting, incubate the cells 1 – 3 minutes at 37°C with recommended volume of indicated trypsinization reagent (please see required material)
1.9 Neutralize trypsinization reaction with TNS once the majority of the cells (>90%) have been detached

2. Nucleofection®

One Nucleofection® Sample contains
0.5 – 1 x 10^6 cells
1 – 5 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 Dermal Fibroblast Nucleofector® Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 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
2.3 Harvest the cells by trypsinization (please see 1.7 – 1.9)
2.4 Count an aliquot of the trypsinized cells and determine cell density
2.5 Centrifuge the required number of cells (**0.5 – 1 x 10^6 cells per sample**) at **200xg for 10 minutes** at room temperature
2.6 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample

Note **Avoid storing the cell suspension for longer than 15 minutes in Nucleofector® Solution as this reduces cell viability and transfection efficiency.**

2.7 Combine 100 µl of cell suspension with **1 – 5 µg DNA**, 2 µg pmaxGFP® Vector or **30 nM – 300 nM** siRNA (3 – 30 pmol/sample) or other substrates
2.8 Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles. Close the cuvette with the cap
2.9 Select the appropriate Nucleofector® Program **U-020 (U-20 for Nucleofector® I Device)**
2.10 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.11 Take the cuvette out of the holder once the program is finished
2.12 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 is often detectable already after 4 – 5 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:

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References

1. Frolov A, Zielinski et al, J Biol Chem. 2003;278(28):25517-25.
2. Javelaud D et al, J Biol Chem. 2003;278(27):24624-8.
3. Neff L et al, J Biol Chem. 2003;278(30):27721-8.
4. Ohtani N et al, J Cell Biol. 2003;162(2):173-83.

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Please note that the Amaxa® Nucleofector® Technology is not intended to be used for diagnostic purposes or for testing or treatment in humans.

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