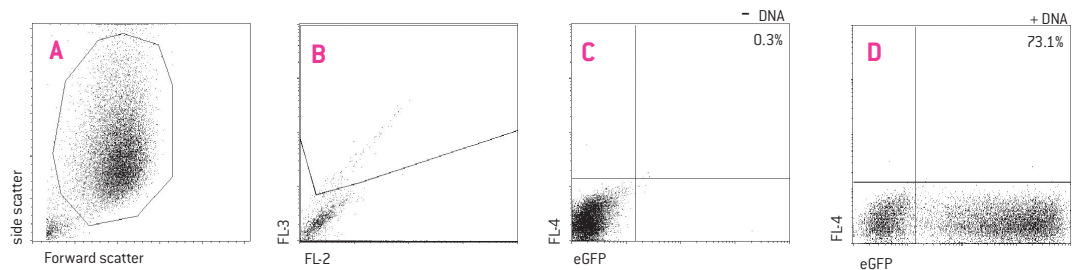


Amaxa[®] Human Dermal Fibroblast Nucleofector[®] Kit

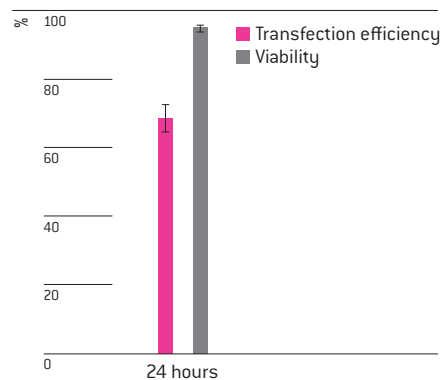
For Normal Human Dermal Fibroblasts - Adult (NHDF-Adult)

Validated to work with Clonetics[®] NHDF-Ad [Lonza; Cat. No. CC-2511]; adherent fibroblastoid cell

Example for Nucleofection[®] of NHDF-Adult



NHDF-Adult were transfected using the Human Dermal Fibroblast Nucleofector[®] Kit and a plasmid encoding the fluorescent protein eGFP. 24 hours post Nucleofection[®] cells were analyzed by flow cytometry. NHDF-Adult were gated according to forward/side scatter (A). Dead cells were excluded by staining with propidium iodide and gating (B). eGFP expression of NHDF-Adult is shown after Nucleofection[®] without (C) and with plasmid DNA (D).



Transfection efficiency and viability of NHDF-Adult 24 hours post Nucleofection[®]. Cells were transfected with program U-023 and 2 µg of a plasmid encoding eGFP.

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 40ml aliquots of the 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 (4 – 5 x 10⁵ cells per sample)
Minimal cell number: 2 x 10⁵ cells (a lower cell number may lead to a major decrease of cell viability)
Maximal cell number: 5 x 10⁵ cells

1. Pre Nucleofection®

Note Transfection results may be donor – dependent.

Cell culture recommendations

- 1.1 Seeding conditions: at least 2 x 10⁵ cells/75cm² flask
- 1.2 Replace media every 2 days; 2 – 3 ml media per 25 cm² flask
- 1.3 Cells should be passaged after reaching 80 – 90% confluency
- 1.4 For Nucleofection® cells should be preferably passaged 6 days before
- 1.5 Do not use cells after passage number 9 as this may result in substantially lower gene transfer efficiency and viability
- 1.6 Optimal confluency before Nucleofection® 80 – 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

4 – 5 x 10 ⁵ cells
2 – 3 µg plasmid DNA (in 1 – 5 µl H ₂ O 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% CO₂ 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 (**4 – 5 x 10⁵ 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. 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 **2 – 3 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-023** for high transfection efficiency or **P-022** for high cell viability (**U-23** or **P-22** 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% CO₂ incubator until analysis. Gene expression is often detectable already 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

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References

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2. Javelaud D et al, J Biol Chem. 2003;278(27):24624-8.
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4. Ohtani N et al, J Cell Biol. 2003;162(2):173-83.

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