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).

**Product Description**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>VPD-1001</th>
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<tbody>
<tr>
<td>Size (Reactions)</td>
<td>25</td>
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<tr>
<td>Human Dermal Fibroblast Nucleofector® Solution</td>
<td>2.25 ml (2.05 ml + 10% overfill)</td>
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<tr>
<td>Supplement</td>
<td>0.5 ml (0.45 ml + 10% overfill)</td>
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<tr>
<td>pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)</td>
<td>30 µg</td>
</tr>
<tr>
<td>Certified cuvettes</td>
<td>25</td>
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<tr>
<td>Plastic pipettes</td>
<td>25</td>
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</table>

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/25 cm² 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 H₂O 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/equilibr IReadOnly to 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 (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% CO₂ incubator until analysis. Gene expression is often detectable already after 4 – 5 hours
Optimized Protocol for Normal Human Dermal Fibroblasts - Neonatal (NHDF-Neo)

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


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