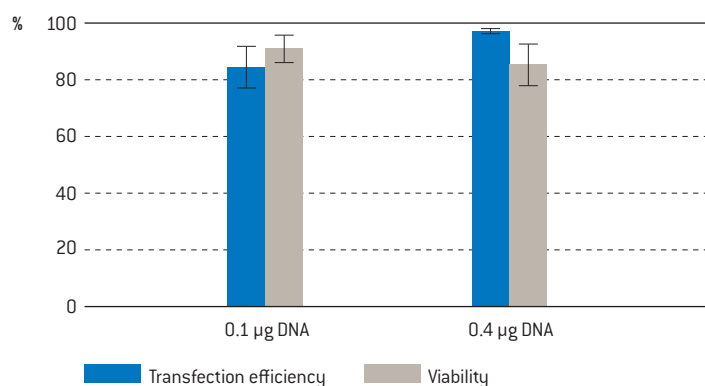


Amaxa™ 96-well Shuttle™ Protocol for Normal Human Dermal Fibroblasts – Neonatal

Cell Description

This protocol has been validated for Clonetics™ NHDF-Neo (Lonza; Cat. No. CC-2509); adherent fibroblastoid cells.

Example for 96-well Nucleofection™ of fibroblast cells



Transfection efficiencies and viabilities of NHDF-Neo 24 hours post Nucleofection™. NHDF-Neo cells were transfected with program 96-DT-130 and 0.1 µg or 0.4 µg of pmaxGFP™ Vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ with HTS option (Becton Dickinson). Cell viability was determined as % PI negative cells.

Product Description

Recommended Kits

P2 Primary Cell 96-well Nucleofector™ Kits

Cat. No.	V4SP-2096
Size (reactions)	1×96
P2 Primary Cell 96-well Nucleofector™ Solution	2.25 ml
Supplement	0.5 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	1
Cat. No.	V4SP-2960
Size (reactions)	10×96
P2 Primary Cell 96-well Nucleofector™ Solution	22.5 ml
Supplement	5 ml
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
Nucleocuvette™ Plate (s)	10

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 expiry 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.

Note

96-well Nucleofector™ Solutions can only be used with conductive polymer cuvettes, i.e. in the 96-well Shuttle™ Device and in the 4D-Nucleofector™ System. They are not compatible with the Nucleofector™ II/2b Device.

Required Material

Note

Please make sure that the entire supplement is added to the Nucleofector™ Solution.

- Nucleofector™ 96-well Shuttle System (Nucleofector™ Device, version IIS; 96-well Shuttle™ Device; laptop with 96-well Shuttle™ Software)
- Supplemented 96-well Nucleofector™ Solution at room temperature
- Supplied Nucleocuvette™ Plate(s)
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µl

Note

Volume of substrate solution added to each sample should not exceed 10 % of the total reaction volume (2 µl for 20 µl reactions). For positive control using pmaxGFP™ Vector, please dilute the stock solution to reach the appropriate working concentration.

- Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260 : A280 ratio should be at least 1.8
- 96-well culture plates or culture plates of your choice
- Nucleocuvette™ compatible tips: epT.I.P.S.™ (US/CDN: Eppendorf North America, Cat. No. 2491.431, Rest of World: Eppendorf AG, Cat. No. 0030073.266), Matrix TallTips™ (Matrix Technologies Corp., Cat. No. 7281) or LTS Tips (Rainin Instrument, LLC, Cat. No. SR-L10F, SR/SS-L250S, SR/SS-L300S). Before using other types of pipette tips, please ensure they reach the bottom of the Nucleocuvette™ Wells without getting stuck
- 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 media to 37°C (255 µl per sample)
- Appropriate number of cells (5×10⁴–1×10⁵ cells per sample)

1. Pre Nucleofection™

Note

Transfection results may be donor-dependent.

Cell Culture Recommendations

- 1.1 Seeding conditions: At least 5×10⁴ 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

- 5×10^4 – 1×10^5 cells
- 0.1–1 µg plasmid DNA (in 1–2 µl H₂O or TE) or 0.4 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P2 Primary Cell 96-well Nucleofector™ Solution

- 2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
- 2.2 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see Manual “Nucleofector™ 96-well Shuttle™ System”)
- 2.3 Select the appropriate Nucleofector™ Program **96-DT-130**
- 2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 175 µl for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5% CO₂ incubator
- 2.5 Pre-warm an aliquot of culture medium to 37°C (80 µl per sample* see note at the end of this chapter)
- 2.6 Prepare 0.1–1 µg plasmid DNA or 0.4 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30 nM–300 nM siRNA (0.6–6 pmol/sample)
- 2.7 Harvest the cells by trypsinization (please see 1.7–1.9)
- 2.8 Count an aliquot of the trypsinized cells and determine cell density
- 2.9 Centrifuge the required numbers of cells (5×10^4 – 1×10^5 cells per sample) at 90×g for 10 minutes at room temperature
- 2.10 Resuspend the cell pellet carefully in 20 µl room temperature 96-well Nucleofector™ Solution per sample

A: One or several substrates (DNAs or RNAs) in multiples

- Prepare mastermixes by dividing cell suspension according to number of substrates
- Add required amount of substrates to each aliquot (max. 2 µl per sample)
- Transfer 20 µl of mastermixes into the wells of the 96-well Nucleocuvette™ Modules

B: Multiple substrates (e.g. Library Transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U- or V-bottom 96-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 96-well Nucleocuvette™ Modules

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to pipet from a pipetting reservoir for multi-channel pipettes. Use a multi-channel or single-channel pipette with suitable pipette tips. As leaving cells in 96-well Nucleofector™ Solution for extended periods of time may lead to reduced transfection efficiency and viability it is important to work as quickly as possible. Avoid air bubbles while pipetting.

- 2.11 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well
- 2.12 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle™. Well “A1” must be in upper left position
- 2.13 Start 96-well Nucleofection™ Process by either pressing “Upload and start” in the 96-well Shuttle™ Software or pressing “Upload” in the 96-well Shuttle™ Software and then the “Start” button at the 96-well Shuttle™ (for both options please refer to the respective Manual)
- 2.14 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer
- 2.15 Incubate Nucleocuvette™ Plate 10 minutes at room temperature
- 2.16 Resuspend cells with desired volume of pre-warmed medium (maximum cuvette volume 200 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 80 µl of pre-warmed medium*
- 2.17 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 25 µl of resuspended cells to 175 µl pre-warmed media prepared in 96-well culture plates*

* Note

The indicated cell numbers and volumes have been found to produce optimal 96-well Nucleofection™ Results in most cases, however, depending on your specific needs you may wish to test an extended range of cell numbers. Cell numbers and volumes can be adapted such that fewer cells are transferred or duplicate plates can be seeded.

3. Post Nucleofection™

- 3.1 Incubate the cells in a humidified 37°C/5% CO₂ incubator until analysis. Gene expression is often detectable after only 4–8 hours

Additional Information

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

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