BioResearch

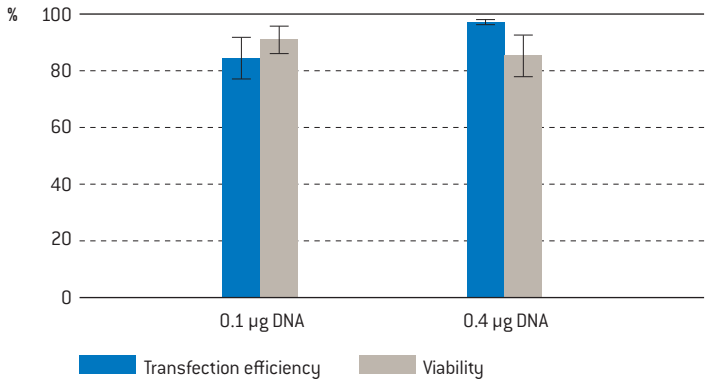
Lonza

Amaxa™ HT Nucleofector™ 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 Nucleofection™ of fibroblast cells



Transfection efficiencies and viabilities of NHDF-Neo 24 hours post Nucleofection™. NHDF-neo cells were transfected with program DT-130-AA 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 determinded as % PI negative cells.

Product description

Recommended kits

P2 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-2002
Size (reactions)	2×384
P2 primary cell HT Nucleofector™ solution	22.20 ml
Supplement	5.0 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-2010
Size (reactions)	10×384
P2 primary cell HT Nucleofector™ solution	90 ml
Supplement	20 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well 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

HT Nucleofector™ solutions can only be used with conductive polymer cuvettes, i.e. in the HT Nucleofector™, 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.

- HT Nucleofector™ System
- Supplemented HT Nucleofector™ solution at room temperature
- Supplied 384-well 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
- 384-well Nucleocuvette™ plates are best handled with an automated liquid handling system. If manual pipetting is required please use 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 (225 µl per sample)
- Appropriate number of cells (5×10^4–1×10^5 cells per sample)

1. Pre Nucleofection™

Note
Transfection results may be donor-dependent.

Cell culture recommendations

1.1 Seeding conditions: At least 5×10^4 cells/25cm^2 flask
1.2 Replace media 2–3 times per week; 2–3 ml media per 25 cm^2 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 H_2O or TE) or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P2 primary cell HT Nucleofector™ solution

2.1 Please make sure that the entire supplement is added to the Nucleofector™ solution

2.2 Start HT Nucleofector™ software, verify device connection and upload experimental parameter file (for details refer to HT Nucleofector™ manuals)

2.3 Select the appropriate HT Nucleofector™ program DT-130-AA

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 185 µl for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator

2.5 Pre-warm an aliquot of culture medium to 37°C (40 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 HT 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 384-well Nucleocuvette™ plate

B: Multiple substrates (e.g. library transfection)

- Pipette 20 µl of cell suspension into each well of a sterile U-bottom 384-well microtiter plate
- Add 2 µl substrates (maximum) to each well
- Transfer 20 µl of cells with substrates into the wells of the 384-well Nucleocuvette™ plate

Note

It is advisable to pre-dispense each cell suspension into a sterile round-bottom 384-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 HT 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 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate

2.12 Place 384-well Nucleocuvette™ plate with closed lid onto the carousel of the plate handler of the HT Nucleofector™. Well "A1" must be in upper left position

2.13 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software (for details refer to HT Nucleofector™ manuals)

2.14 After run completion, carefully remove the 384-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 volume 60 µl). Mix cells by gently pipetting cells up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40 µl of pre-warmed medium*

2.17 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 15 µl of resuspended cells to 185 µl pre-warmed media prepared in 96-well culture plates*

*** Note**

The indicated cell numbers and volumes have been found to produce optimal 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 % CO_2 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

Technical assistance and scientific support

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