BioResearch

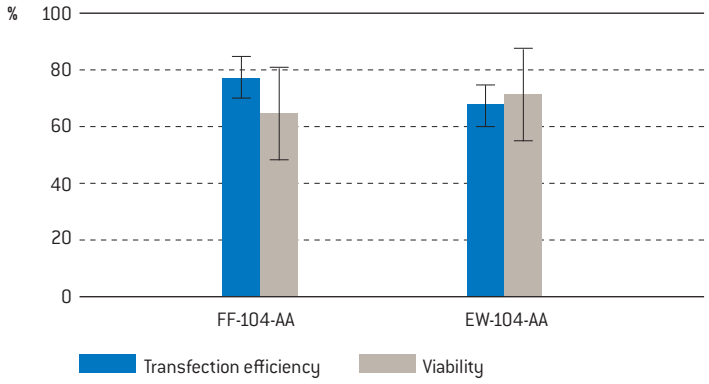
Lonza

Amaxa™ HT Nucleofector™ protocol for human mesenchymal stem cells (MSC)

Cell description

This protocol has been validated for self isolated or Poietics™ human mesenchymal stem cells from bone marrow (Lonza, Cat. No. PT-2501)

Example for Nucleofection™ of human MSCs



Average transfection efficiency and viability of hMSC 24 hours post Nucleofection™. Expanded 5×10^4 hMSC (Lonza, Cat. No. PT-2501) were transfected with program FF-104-AA or EW-104-AA and 0.4 µg of pmaxGFP™ vector. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ (Becton Dickinson) with HTS option. Cell viability was determined as a relative portion of untreated control. (measured with the ViaLight™ Plus Bioassay Kit; Lonza, Cat. No. LT07-221).

Product description

Recommended kits

P1 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-1002
Size (reactions)	2×384
P1 primary cell HT Nucleofector™ solution	22.5 ml
Supplement	5 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg
384-well Nucleocuvette™ plate(s)	2

Cat. No.	V5SP-1010
Size (reactions)	10×384
P1 primary cell HT Nucleofector™ solution	90ml
Supplement	20 ml
pmaxGFP™ vector (1 µg/µl in 10 mM Tris pH 8.0)	150 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 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.

Note

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

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
- Culture medium: MSCGM™ Mesenchymal Stem Cell Growth Medium BulletKit™(Lonza, Cat. No. PT-3001)
- Differentiation medium (for adipogenic differentiation): hMSC Mesenchymal Stem Cell Adipogenic Differentiation BulletKit™ (Lonza, Cat. No. PT-3004)
- Differentiation medium (for osteogenic differentiation): hMSC Mesenchymal Stem Cell Osteogenic Differentiation BulletKit™ (Lonza, Cat. No. PT-3002)
- Differentiation medium (for chondrogenic differentiation): hMSC Mesenchymal Stem Cell Chondrogenic Differentiation BulletKit™ (Lonza, Cat. No. PT-3003)
- Trypsin/EDTA (Lonza, Cat. No. CC-3232)
- PBS/BSA: PBS containing 0.5 % BSA
- Pre-warm appropriate volume of culture medium to 37°C (113 µl per sample)
- Appropriate number of cells (5×10^4 cells) per sample; lower or higher cell numbers may influence transfection results)

1. Pre Nucleofection™

Note
Cells may be expanded in culture medium (please see cell culture recommendations below). However, it is recommended to use early passages (<P9). Transfection results may be donor-dependent.

Cell culture recommendations

1.1 Replace media every 2–3 days
1.2 For passaging trypsinize cells as recommended by supplier, stop trypzinization by adding PBS/BSA, spin cells down and resuspend them in fresh media. Passage cells 1–2 times a week
1.3 Make sure that cells do not grow confluent during expansion
1.4 Seed out $2.5\times10_5$ cells / T75 cm^2 for expansion

Note
For preparation of self isolated human mesenchymal stem cells please follow the respective

Trypsinization

1.5 Wash the attached cell layer with Dulbecco's Phosphate Buffered Saline or an equivalent calcium and magnesium free balanced salt solution
1.6 Add a sufficient volume of Trypsin-EDTA solution to cover the cell layer (approx. 0.05 ml/cm^2), and gently swirl the dish/flask to ensure an even distribution of the solution
1.7 Incubate at room temperature for five minutes, then observe under a microscope to avoid overexposure of cells to trypsin. If the cells are less than 90 % detached, continue incubating and observe every 3 minutes. Tapping the flask or plate will expedite cell detachment. Do not incubate the cells longer than 15 minutes. If necessary, prolong the incubation time for two more minutes at 37°C
1.8 Once the majority of cells (>90 %) have been dislodged, add an equal volume of PBS or temperature equilibrated medium to the dish/flask. Disperse the solution by pipetting over the cell layer surface several times
1.9 To remove the trypsin, centrifuge cells at approximately 600×g for five minutes at room temperature

2. Nucleofection™

One Nucleofection™ sample contains

- 5×10^4 cells
- 0.2–1 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 P1 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 the HT Nucleofector™ manuals)

2.3 Select the appropriate HT Nucleofector™ program **FF-104-AA** (high efficiency) or **EW-104-AA** (high viability)

2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 73 µl* (see note at the end of this chapter) for one well of a 96-well plate and preincubate/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, 10 µl overfill)

2.6 Prepare 0.2–1 µg plasmid DNA or 0.4 µg pmaxGFP™ vector or 30–300 nM siRNA (0.6–6 pmol/sample)

2.7 Count an aliquot of the cells and determine cell density

2.8 Centrifuge the required number of cells (5×10^4 cells per sample) at 200×g for 10 minutes at room temperature. Remove supernatant completely

2.9 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 liquid handling system or at least a multi-channel pipette with suitable pipette tips. Avoid air bubbles while pipetting.

2.10 Briefly shake the 384-well Nucleocuvette™ plate with an apprpriate microtiter plate shaker to make sure the sample bottom of the wells and avoid air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate

2.11 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

2.12 Start Nucleofection™ process clicking "Start" in the HT Nucleofector™ software

2.13 After run completion, carefully remove the 384-well Nucleocuvette™ plate from the carousel

2.14 Incubate plate for 10 minutes at room temperature

2.15 Resuspend cells with desired volume of pre-warmed culture medium I (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 384-well plates: Resuspend cells in 40 µl of pre-warmed media*

2.16 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 27 µl of resuspended cells to 73 µl pre-warmed medium 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 humidified 37°C/5 % CO_2 incubator until analysis. Gene expression or down regulation, respectively, is often detectable after only 4–8 hours. For long-term analysis, we recommend changing the medium 24 hours post Nucleofection™.

3.2 For differentiation of human mesenchymal stem cells, you may plate cells directly in the respective differentiation medium (see required material section) post Nucleofection™. Depending on the kind of differentiation, cell numbers should be adjusted as recommended (e.g. for adipogenic differentiation cells should be highly confluent before induction (see also Lonza protocols); for osteogenic differentiation cells have to be plated in low cell number before induction (Lonza protocols))

Note

Results could be differ between different donors of cells.

Additional information

Up-to-date List of all Nucleofector™ references

www.lonza.com/nucleofection-citations

Technical assistance and scientific support

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References

1. Mok et al., 2008 (Cytotherapy)
2. Wiehe et al. 2007 (J Cell Mol Med)
3. Aslan et al., 2006 (Tissue Eng)
4. Haleem-Smith et al., 2005 (Mol Biotechnol)

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