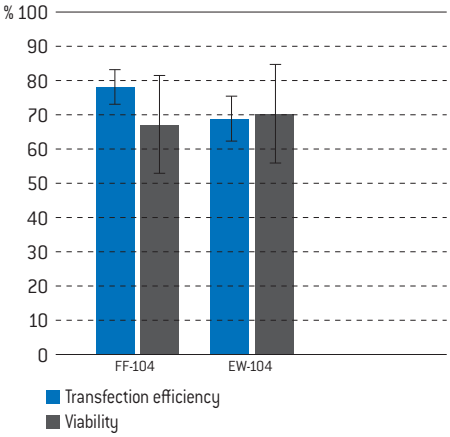
BioResearch

Amaxa™ 4D-Nucleofector™ Protocol for Undifferentiated Human Mesenchymal Stem Cells [MSC]

For 4D-Nucleofector™ X Unit—Transfection in suspension

Self-isolated or Poietics® Human Mesenchymal Stem Cells from bone marrow [Lonza, Cat. No. PT-2501]



Example for Nucleofection™ of human mesenchymal stem cells

Average transfection efficiency and viability of human mesenchymal stem cells (hMSC) 24 hours post Nucleofection™. Expanded 5 x 10^4 hMSC [Lonza, Cat. No. PT-2501] were transfected with program FF-104 or EW-104 and 0.4 µg of pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. 24 hours post Nucleofection™ cells were analyzed on a FACSCalibur™ (Becton Dickinson). 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 Kit(s)—P1 Primary Cell 4D-Nucleofector™ X Kit

Cat. No.	V4XP-1012	V4XP-1024	V4XP-1032
Transfection volume	100 µl	100 µl	20 µl
Size [reaction]	2 x 6	24	2 x 16
Nucleofector™ Solution	2 x 0.675 ml (0.492 ml + 27% overfill)	2.25 ml (1.968 ml + 13% overfill)	0.675 ml (0.525 ml + 22% overfill)
Supplement	2 x 0.15 ml (0.108 ml + 27% overfill)	0.5 ml (0.432 ml + 13% overfill)	0.15 ml (0.115 ml + 22% overfill)
pmaxGFP™ Vector (1 µg/µl in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg
Single Nucleocuvette™ (100 µl)	12	24	-
16-well Nucleocuvette™ Strips (20 µl)	-	-	2

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

4D-Nucleofector™ Solutions can only be used with Nucleovettes™ (conductive polymer cuvettes), i.e. in the 4D-Nucleofector™ System and the 96-well Shuttle™ Device. 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. The ratio of Nucleofector™ Solution to supplement is 4.5 : 1 (see table 1)

- 4D-Nucleofector™ System (4D-Nucleofector™ Core Unit and 4D-Nucleofector™ X Unit)
- Supplemented 4D-Nucleofector™ Solution at room temperature
- Supplied 100 µl single Nucleocuvette™ or 20 µl 16-well Nucleocuvette™ Strips
- **Compatible tips for 20 µl Nucleocuvette™ Strips:** 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 Instruments, 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
- **Supplied pmaxGFP™ Vector, stock solution 1 µg/µl**

Note
For positive control using pmaxGFP™, dilute the stock solution to an appropriate working concentration. Further details are provided in table 3 of this Optimized Protocol. The volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µl for 20 µl reactions; 10 µl for 100 µl reactions).

- Substrate of interest, highly purified, preferably by using endotoxin-free kits; A260:A280 ratio should be at least 1.8
- Cell culture plates of your choice
- **For detaching cells:** Trypsin/EDTA (Lonza, Cat. No. CC-3232)
- **Culture medium:** MSCGM™ Mesenchymal Stem Cell Growth Medium BulletKit™[Lonza, Cat. No. PT-3001]
- **Differentiation medium (for adipogenic differentiation post Nucleofection™):** hMSC Mesenchymal Stem Cell Adipogenic Differentiation BulletKit™ [Lonza, Cat. No. PT-3004]
- **Differentiation medium (for osteogenic differentiation post Nucleofection™):** hMSC Mesenchymal Stem Cell Osteogenic Differentiation BulletKit™ [Lonza, Cat. No. PT-3002]
- **Differentiation medium (for chondrogenic differentiation post Nucleofection™):** hMSC Mesenchymal Stem Cell Chondrogenic Differentiation BulletKit™ [Lonza, Cat. No. PT-3003]
- PBS/BSA: PBS containing 0.5% BSA
- Prewarm appropriate volume of culture medium to 37°C (see table 2)
- Appropriate number of cells/sample (see table 2)

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 x 10^5 cells / T75 cm^2 for expansion

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

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 x g for five minutes at room temperature

2. Nucleofection™

For Nucleofection™ Sample contents and recommended Nucleofector™ Program, please refer to Table 3.

2.1 Please make sure that the entire supplement is added to the Nucleofector™ Solution
2.2 Start 4D-Nucleofector™ System and create or upload experimental parameter file (for details see device manual)

2.3 Select/Check for the appropriate Nucleofector™ Program (see table 3)
2.4 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media (see table 4) 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 (see table 4)
2.6 Prepare plasmid DNA or pmaxGFP™ Vector or siRNA (see table 3)
2.7 Harvest the cells by trypsinization (see 1.5–1.8)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (see table 3) at 200xg for 10 minutes at room temperature. Remove supernatant completely
2.10 Resuspend the cell pellet carefully in room temperature 4D-Nucleofector™ Solution (see table 3)
2.11 Prepare mastermixes by dividing cell suspension according to number of substrates
2.12 Add required amount of substrates to each aliquot (max. 10% of final sample volume)
2.13 Transfer mastermixes into the Nucleocuvette™ Vessels

Note
As leaving cells in 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.

2.14 Gently tap the Nucleocuvette™ Vessels to make sure the sample covers the bottom of the cuvette
2.15 Place Nucleocuvette™ Vessel with closed lid into the retainer of the 4D-Nucleofector™ X Unit. Check for proper orientation of the Nucleocuvette™ Vessel
2.16 Start Nucleofection™ Process by pressing the "Start" on the display of the 4D-Nucleofector™ Core Unit (for details, please refer to the device manual)
2.17 After run completion, carefully remove the Nucleocuvette™ Vessel from the retainer
2.18 Incubate Nucleocuvette™ 10 minutes at room temperature
2.19 Resuspend cells with pre-warmed medium (for recommended volumes see table 5). Mix cells by gently pipetting up and down two to three times. When working with the 100 µl Nucleocuvette™ use the supplied pipettes and avoid repeated aspiration of the sample
2.20 Plate desired amount of cells in culture system of your choice (for recommended volumes see table 5)

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 differ between different donors of cells.

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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3. Aslan H et al. (2006) Tissue Eng 12(4):877-89
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Table 1: Volumes required for a single reaction

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Volume of Nucleofector™ Solution	82 µl	16.4 µl
Volume of Supplement	18 µl	3.6 µl

Table 2: Required amounts of cells and media for Nucleofection™

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Culture medium per sample post Nucleofection™ (for transfer and culture)	1.5 ml	140 µl
Cell number per Nucleofection™ Sample	4–5 x 10^5 (Minimal cell number: 2 x 10^5 cells, a lower cell number may lead to a major increase in cell mortality; maximum cell number: 6 x 10^5)	5 x 10^4 cells (Lower or higher cell numbers may influence transfection results)

Table 3: Contents of one Nucleofection™ Sample and recommended program

		100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip
Cells		4–5 x 10^5	5 x 10^4
Substrate*	**pmaxGFP™ Vector**	2 µg	0.4 µg
or	**plasmid DNA (in H_2O or TE)**	2 µg	0.2–1 µg
or	**siRNA**	30–300nM siRNA (3–30 pmol/sample)	30–300nM siRNA (0.6–6 pmol/sample)
P1 Primary Cell 4D-Nucleofector™ X Solution		100 µl	20 µl
Program		FF-104 (high efficiency) EW-104 (high viability)	FF-104 (high efficiency) EW-104 (high viability)

* Volume of substrate should comprise maximum 10% of total reaction volume

Table 4: Culture volumes required for post Nucleofection™ Steps

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
6-well culture plate	1 ml	-
96-well culture plate	-	50 µl
Culture medium to be added to the sample post Nucleofection™	500 µl	90 µl

* Maximum cuvette volume 200 µl

Table 5: Recommended volumes for sample transfer into culture plate

	100 µl Single Nucleocuvette™	20 µl Nucleocuvette™ Strip*
Culture medium to be added to the sample post Nucleofection™	500 µl	90 µl
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	50 µl

* Maximum cuvette volume 200 µl