BioResearch

Lonza

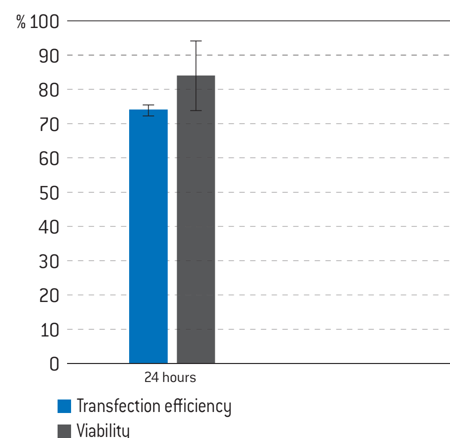
Amaxa™ 4D-Nucleofector™ Protocol for Primary Human Chondrocytes

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

Primary human adult chondrocytes obtained from articular cartilage 24–72 hours post mortem; fibroblastoid, but not roundish, spindle like cells

Example for Nucleofection™ of primary human chondrocytes

Transfection efficiency of human chondrocytes 24 hours post Nucleofection™. 2 x 10^5 cells were transfected with program ER-100 using 1.0 µg pmaxGFP™ Vector in 20 µl Nucleocuvette™ Strips. Post Nucleofection™ 1.8 x 10^5 cells were seeded in one well of a 96-well plate. Cells were analyzed 24 hours post Nucleofection™ for GFP expression using a Becton Dickinson FACSCalibur™. Viability Cell viability was measured by using the CellTiter-Glo® assay (Promega). Cell viability is given in percent compared to non-transfected control.



Product Description

Recommended Kit(s)—P3 Primary Cell 4D-Nucleofector™ X Kit

Cat. No.	V4XP-3012	V4XP-3024	V4XP-3032
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 1µg/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
- **Culture medium:** DMEM/F-12 (1 : 1) [Lonza; Cat. No. 12-719F] supplemented with 10 % FCS, 50µg/ml 2-Phospho-L-ascorbic acid trisodium salt [Fluka, Cat.-No. 49752] and Penicillin/Streptomycin (Penicillin: 50 U/ml; Streptomycin: 50 µg/ml)
- **Isolation medium:** DMEM/F-12 (1 : 1) [Lonza; Cat. No. 12-719F] supplemented with 250ng/ml Fungizone® Antimycotic [Invitrogen, Cat.-No. 15290-026] and Penicillin/Streptomycin (Penicillin: 50 U/ml; Streptomycin: 50 µg/ml)
- **Pronase solution:** Resuspend pronase [Roche, Cat. No. 1459643] in culture medium at a final concentration of 1.0 mg/ml and sterilize by filtration (prepare 40 ml of pronase solution for up to 15 g cartilage tissue, 60 ml are required if more than 15 g cartilage tissue will be used)
- **Collagenase solution:** Resuspend collagenase [Serva, Cat. No. 17465] in DMEM/F-12 medium at a final concentration of 1 mg/ml and sterilize by filtration (prepare 40 ml of collagenase solution for up to 15g cartilage tissue, 60 ml are required if more than 15 g cartilage tissue will be used)
- **Collagenase/Pronase solution:** Resuspend collagenase [Serva, Cat. No. 17465] and pronase [Roche, Cat. No. 1459643] at a concentration of 1 mg/ml each in culture medium. Pass solution through a sterile filter. Use 10 ml of this solution per 10 cm culture dish
- Prewarm appropriate volume of culture medium to 37 °C (see table 2)
- Appropriate number of cells/sample (see table 2)

1. Pre Nucleofection™

Note

Transfection results may be donor-dependent.

Preparation of human chondrocytes

We strongly recommend isolating chondrocytes by pronase/collagenase treatment as follows:

1.1 Withdraw cartilage tissue under sterile conditions and transfer the tissue into isolation medium
1.2 Cut the cartilage tissue into pieces of approximately 2 x 2 mm (preferably in a glass petri dish) and transfer them afterwards into a sterile 250 ml glass bottle (weigh empty bottle)
1.3 Wash cartilage pieces twice with PBS
1.4 Add 40 ml pronase solution and shake the cartilage pieces for 30 minutes at 37 °C (100–120 rpm)
1.5 Incubate the cartilage with collagenase solution for 18–(see 2–3 days) 24 hours at 37 °C with slow agitation (100–120 rpm)
1.6 Filtrate the cell suspension through a 70 µm filter into 50 ml falcon tubes
1.7 Centrifuge the filtered cell suspension at room temperature for 10 minutes (300xg)
1.8 Discard supernatant carefully and wash cell pellet twice with PBS
1.9 Resuspend cells in an appropriate volume (20–50 ml) of culture medium carefully
1.10 Take an aliquot of the cell suspension (10 µl) and mix it with 90 µl trypan blue to count the cells

Note

The digestion should be as complete as possible. Incomplete digestion will decrease the quality of the cultured chondrocytes and reduce the nucleofection performance. The digest has been performed properly if the vast majority of chondrocytes does not have an external matrix. In addition most cells should be adherent 12–24 hours post seeding.

Cultivation of chondrocytes

1.11 In order to cultivate chondrocytes in high density monolayers, 1.8×10^5 cells are seeded per cm^2. We recommend using 10 cm culture dishes
1.12 Cultivate cells in high density culture for 2–3 days

Detaching chondrocytes for Nucleofection™

1.13 Take the cultivated chondrocytes and aspirate the culture medium 4 hours before Nucleofection™
1.14 Wash cells once with PBS
1.15 Add pronase/collagenase solution (10 ml per 10 cm^2 culture dish) and incubate the chondrocytes for 3–5 hours at 37 °C

Note

This incubation with pronase/collagenase step is necessary to detach the cells and to remove extracellular matrix. Cells will detach quite fast but removing the extracellular matrix takes several hours. Cells surrounded by extracellular matrix may form clumps and can be identified by their roundish shape. Singularizing cells by this long incubation with pronase/collagenase improves the Nucleofection™ Performance remarkably. You may improve this procedure by pipetting the cell suspension once per hour.

1.16 After the collagenase/pronase treatment chondrocytes can easily be rinsed off the substrate

2. Nucleofection™

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 5) 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 Detach the cultivated chondrocytes by promise/collagenase treatment 4 hours before Nucleofection™ (see 1.13–1.16)
2.8 Count an aliquot of the cells and determine cell density
2.9 Centrifuge the required number of cells (see table 3) at 300xg 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
3.2 Change medium after 24 hours

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

1. Human Chondrocyte Culture as Models of Cartilage Specific Gene Regulation; Methods in Molecular Medicine 107, 69-95; Human Cell Culture Protocols; Second Edition; Humana Press Inc., Totowa, NJ

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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	200 µl
Cell number per Nucleofection™ Sample	5×10^{5}–1×10^{6} cells (Lower or higher cell numbers may influence transfection results)	2×10^{5} 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			5×10^{5}–1×10^{6}	2×10^{5}
Substrate*		**pmaxGFP™ Vector**	2 µg	1 µg
	or	**plasmid DNA (in H_2O or TE)**	2–5 µg	1–2 µg
	or	**siRNA**	30–300nM siRNA (3–30 pmol/sample)	30–300nM siRNA (0.6–6 pmol/sample)
P3 Primary Cell 4D-Nucleofector™ X Solution			100 µl	20 µl
Program			ER-100	ER-100

* 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	-
12-well culture plate	-	-
96-well culture plate	-	20 µl
Culture medium to be added to the sample post Nucleofection™	500 µl	180 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	180 µl
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	180 µl

* Maximum cuvette volume 200 µl