BioResearch

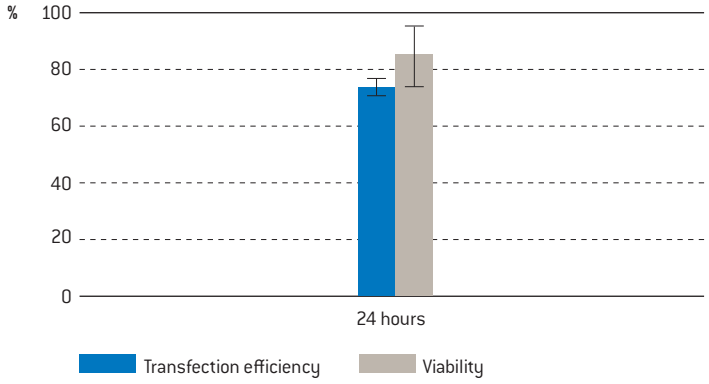
Lonza

Amaxa™ 96-well Shuttle™ Protocol for Human Chondrocytes

Cell Description

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

Example for Nucleofection™ of Human Chondrocytes



Transfection efficiency of human chondrocytes 24 hours post Nucleofection™. 2×10^5 cells were transfected with program 96-ER-100 using 1.0 µg pmaxGFP™ Vector, 1.8×10^5 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™. Cell viability was measured by using the CellTiter-Glo™ assay (Promega). Cell viability is given in percent compared to non-nucleofected control.

Product Description

Recommended Kits

P3 Primary Cell 96-well Nucleofector™ Kits"

Cat. No.	V4SP-3096
Size (reactions)	1×96
P3 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-3960
Size (reactions)	10×96
P3 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
- Isolation medium: DMEM/F-12 (1 : 1) (Lonza; Cat. No. 12-719F) supplemented with 250 ng/ml Fungizone™ Antimycotic (Invitrogen, Cat.-No.: 15290-026) and Penicillin/Streptomycin (Penicillin: 50 U/ml; Streptomycin: 50 µg/ml)
- Culture medium: DMEM/F-12 (1 : 1) (Lonza; Cat. No. 12-719F) supplemented with 10 % FCS, 50 µg/ml 2-Phospho-L-ascorbic acid trisodium salt (Fluka, Cat.-No.: 49752) 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 15 g 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 media at 37°C (200 µl per sample)
- Appropriate number of cells (2×10^5 cells per sample)

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 2×2 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 18–24 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 (300×g)
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

- 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
- Cultivate cells in high density culture for 2–3 days

2. Nucleofection™

One Nucleofection™ Sample Contains

- 2×10^5 cells
- 1.0–2.0 µg plasmid DNA (in 1–2 µl H_2O or TE) or 1.0 µg pmaxGFP™ Vector or 30–300 nM siRNA (0.6–6 pmol/sample)
- 20 µl P3 Primary Cell 96-well Nucleofector™ Solution

Note

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

2.1 Start Nucleofector™ 96-well Shuttle™ Software, verify device connection and upload experimental parameter file (for details see Manual "Nucleofector™ 96-well Shuttle™ System")

2.2 Select the appropriate Nucleofector™ Program **96-ER-100**

2.3 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended 2.4 culture media, e.g. 20 µl* (see note at the end of this chapter) for one well of a 96-well plate and pre-incubate/equilibrate plates in a humidified 37°C/5 % CO_2 incubator

2.4 Pre-warm an additional aliquot of culture media to 37°C (180 µl* per sample)

2.5 Prepare 1.0–2.0 µg plasmid DNA or 1.0 µg pmaxGFP™ Vector. For siRNA experiments we recommend to start using 30–300 nM siRNA (0.6–6 pmol/sample).

2.6 Take the cultivated chondrocytes and aspirate the culture medium 4 hours before Nucleofection™

2.7 Wash cells once with PBS

2.8 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.

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

2.10 Wash the cells with PBS and centrifuge (10 minutes, 300×g) the required number of cells (2×10^5 cells per well of the 96-well Nucleocuvette™ Plate)

2.11 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.

2.12 Gently tap the Nucleocuvette™ Plate to make sure the sample covers the bottom of the well

2.13 Place the lid onto the 96-well Nucleocuvette™ Plate

2.14 Place 96-well Nucleocuvette™ Plate with closed lid into the retainer of the 96-well Shuttle. Well "A1" must be in upper left position

2.15 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)

2.16 After run completion, open retainer and carefully remove the 96-well Nucleocuvette™ Plate from the retainer

2.17 Incubate the 96-well Nucleocuvette™ Plate 10 minutes at room temperature

2.18 Resuspend cells with desired volume of pre-warmed culture media (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 180 µl of pre-warmed media*

2.19 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 180 µl of resuspended cells to 20 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 % 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

Up-To-Date List of all Nucleofector™ References

www.lonza.com/nucleofection-citations

Technical Assistance and Scientific Support

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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 1. Culture Protocols; Second Edition; Humana Press Inc., Totowa, NJ

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