BioResearch

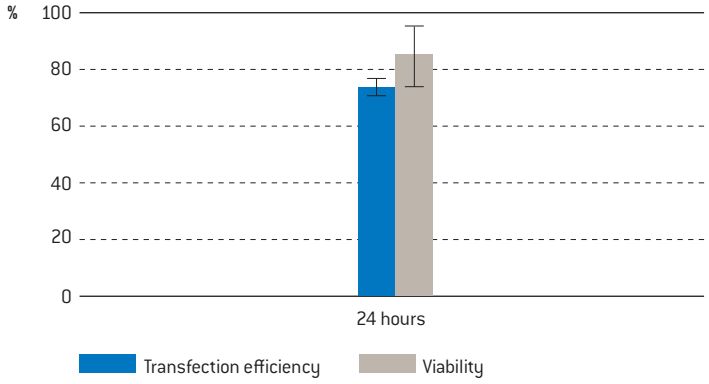
Lonza

Amaxa™ HT Nucleofector™ 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™. $2x10^5$ cells were transfected with program-ER-100-AA using 1.0 µg pmaxGFP™ vector, $1.8x10^5$ 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-transfected control.

Product description

Recommended kits

P3 primary cell HT Nucleofector™ kits

Cat. No.	V5SP-3002
Size (reactions)	2×384
P3 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-3010
Size (reactions)	10×384
P3 primary cell HT Nucleofector™ solution	90 ml
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 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™, 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.

- 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
- 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
- Pre-warm appropriate volume of culture media at 37°C (186 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 $1.8x10^5$ 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

- $2x10^5$ 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 HT Nucleofector™ solution

Note

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

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

2.2 Select the appropriate HT Nucleofector™ program **ER-100-AA**

2.3 Prepare cell culture plates by filling appropriate number of wells with desired volume of recommended culture media, e.g. 146 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 (40 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 xg) the required number of cells ($2x10^5$ cells per well of the 96-well Nucleocuvette™ plate)

2.11 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™ plates

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™ plates

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.

2.12 Briefly shake the 384-well Nucleocuvette™ plate with an appropriate microtiter plate shaker to make sure the sample covers the bottom and the sides of the wells without air bubbles. Alternatively thoroughly tap the 384-well Nucleocuvette™ plate

2.13 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

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

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

2.16 Resuspend cells with desired volume of pre-warmed culture medium (maximum cuvette volume 60 µl). Mix cells by gently pipetting up and down two to three times. Recommendation for 96-well plates: Resuspend cells in 40 µl of pre-warmed media*

2.17 Plate desired amount of cells in culture system of your choice. Recommendation for 96-well plates: Transfer 54 µl of resuspended cells to 146 µl pre-warmed media

* 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 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

4. 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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