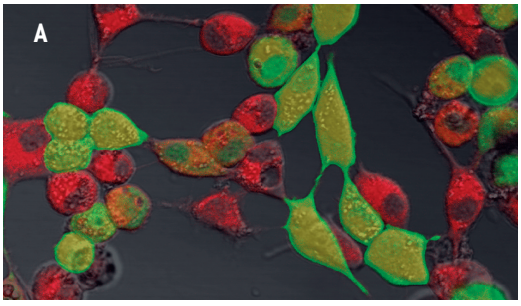
Amaxa® Human Chondrocyte Nucleofector® Kit

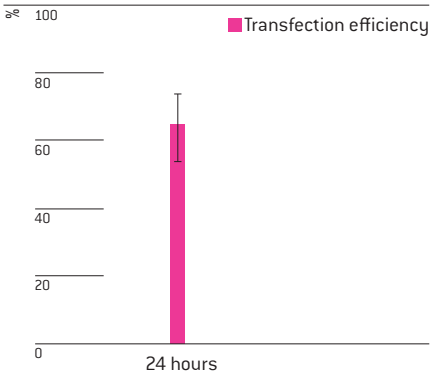
For Primary Human Chondrocytes

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

Example for Nucleofection® of primary human chondrocytes



Example showing typical Nucleofection® results of human chondrocytes. Human chondrocytes were transfected using the Human Chondrocyte Nucleofector® Kit, program U-024 and 5 µg of a plasmid encoding the enhanced green fluorescent protein eGFP. Cell membranes were fluorescently stained in red with the substance R18 (Octadecylrhodamine-B-chloride, Molecular Probes). 24 hours post Nucleofection® the cells were analyzed by fluorescence microscopy. The image shows an overlay of eGFP and R18 fluorescence (Data courtesy of Dr. Schmid and Prof. Aigner, University of Leipzig, Germany).



Average transfection efficiencies of human chondrocytes. Cells were transfected with program U-024 and 5 µg of a plasmid encoding eGFP. 24 hours post Nucleofection®, the cells were analyzed for GFP expression and viability by flow cytometry.

Product Description

Cat. No.	**VPF-1001**
Size (reactions)	25
Cell Line Nucleofector® Solution T	2.25 ml (2.05 ml + 10% overfill)
Supplement	0.5 ml (0.45 ml + 10% overfill)
pmaxGFP® Vector (0.5 µg/µl in 10 mM Tris pH 8.0)	30 µg
Certified cuvettes	25
Plastic pipettes	25
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.

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. For a single reaction use 82 µl of Nucleofector® Solution plus 18 µl of supplement to make 100 µl of total reaction volume.**

- Nucleofector® Device
- Supplemented Nucleofector® Solution at room temperature
- Supplied certified cuvettes
- Supplied plastic pipettes
- Supplied pmaxGFP® Vector
- Substrate of interest, highly purified, preferably by using endotoxin free kits; A260 : A280 ratio should be at least 1.8
- 6-well culture dish or culture system of your choice
- **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)
- **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)
- **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 15g 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 media at 37°C (1.5 ml per sample)
- Appropriate number of cells (5×10^5 – 1×10^6 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 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 to 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 pellets twice with PBS
1.9 Resuspend cells in an appropriate volume (20ml – 50ml) 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 x 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

2. Nucleofection®

One Nucleofection® Sample contains

5 x 10^5 – 1x10^6 cells
2 – 5 µg plasmid DNA (in 1 – 5 µl H_2O or TE) or 2 µg pmaxGFP® or 30-300 nM siRNA (3 – 30 pmol/sample)
100 µl Human Chondrocyte Nucleofector® Solution

2.1 Please make sure that the entire supplement is added to the Nucleofector® Solution
2.2 Prepare 6-well plates by filling appropriate number of wells with 1 ml of supplemented culture media and pre-incubate/equilibrate plates in a humidified 37°C/5% CO_2 incubator
2.3 Take the cultivated chondrocytes and aspirate the culture medium 4 hours before Nucleofection®
2.4 Wash cells once with PBS
2.5 Add pronase/collagenase solution (10 ml per 10cm^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 extra cellular matrix. Cells will detach quite fast but removing the extracellular matrix takes several hours. Cells surrounded by extra cellular 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.**

2.6 After the collagenase/pronase treatment chondrocytes can easily be rinsed off the substrate
2.7 Wash the cells with PBS and centrifuge (10 minutes, 300xg) the required number of cells (5 x 10^5 – 1 x 10^6 cells per well of the 96-well Nucleocuvette® Plate)
2.8 Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample.
2.9 Combine 100 µl of cell suspension with **2 – 5 µg DNA**, 2 µg pmaxGFP® DNA or **30 nM – 300 nM siRNA** (3 – 30 pmol/sample) or other substrates
2.10 Transfer cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles. Close the cuvette with the cap

2.11 Select the appropriate Nucleofector® Program **U-024** or **U-028** (**U-24** or **U-28** for Nucleofector® I Device)
2.12 Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the selected program
2.13 Take the cuvette out of the holder once the program is finished
2.14 Add 500 µl of the pre-equilibrated culture media to the cuvette and gently transfer the sample immediately into the 6-well plate (final volume 1.5 ml media per sample). Use the supplied pipettes and avoid repeated pipetting of the sample

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

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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