BioResearch

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Isolation and Cell Culture of Primary Human Chondrocytes
Technical Reference Guide

General Remarks

This protocol is suited to isolate primary adult human chondrocytes from articular cartilage. The protocol is optimized to work on cells from diff erent donors. However, the use of tissue with severe arthritic damages may lead to reduced performance.

Recommended Culture Media

Isolation Medium
DMEM/F-12 (1:1) (1X) [Invitrogen/Gibco; Cat. No. 31330-038] 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) (1X) [Invitrogen/Gibco Cat. No. 31330-038] 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).

Additional Reagents Required

Pronase Solution
Resuspend pronase [Roche, Cat. No. 1459643] in DMEM/F-12 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 1mg/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 final concentration of 1 mg/ml each in DMEM/F-12 Medium. Pass solution through a sterile filter. Use 10 ml of this solution per 10 cm culture dish.

Cell Isolation and Cultivation of Human Chondrocytes

1. Withdraw cartilage tissue under sterile conditions and transfer it into isolation medium.
2. Cut the cartilage tissue into pieces of approximately 2 x 2 mm (preferably in a petri dish) and transfer them afterwards into a sterile 250 ml glass bottle (weigh empty bottle).
3. Wash cartilage pieces twice with PBS.
4. Add 40 ml pronase solution and shake the cartilage pieces for 30 minutes at 37°C (100 – 120 rpm).
5. Incubate the cartilage with collagenase solution for 18 to 24 hours at 37°C with slow agitation (100 – 120 rpm).
6. Filtrate the cell suspension through a 70 µm fi lter into 50 ml falcon tubes.
7. Centrifuge the fi ltered cell suspension at room temperature for 10 minutes (300 xg).
8. Discard supernatant carefully and wash cell pellets twice with PBS.
9. Resuspend cells in an appropriate volume (ca. 20 ml – 50 ml) of culture medium carefully.
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. The digest has been performed properly if the vast majority of chondrocytes does not have a visible 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 x 105 cells are seeded per cm2. We recommend using 10 cm culture dishes.
- Cultivate cells in high density culture for at least 3 days. You can use chondrocytes cultured for up to 10 days or longer.

Preparing Cells for Nucleofection™

Note

This incubation with pronase/collagenase 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 identifi ed 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. Wash cells once with PBS.
2. Add pronase/collagenase solution (10 ml per 10 cm culture dish) and incubate the chondrocytes for 3 – 5 hours at 37°C.
3. After the collagenase/pronase treatment, chondrocytes can easily be rinsed off the substrate.
4. Wash the cells with PBS and centrifuge (10 minutes, 300 xg) the required number of cells. The chondrocytes are now ready for Nucleofection™. Resuspend them in the appropriate Nucleofector™ Solution at cell density recommended in the Optimized Protocol.

Recommended Literature

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, USA.

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