CHONDROCYTES ISOLATION PROTOCOL

Enzymes

COL H recombinant collagenase class II ACTIVITY ≥30.0 Units/mg (Pz Grassmann)

Preparation of COL G and COL H stock solutions

1. Solution A: Reconstitute COL H, do not exceed 300 U/ml final concentration. Make aliquots of COL H 300 U total and store at -20°C

2. Solution B: dissolve thermolysin in in H2O and filter for sterility, final concentration of 1mg/ml. Make aliquots of Thermolysin 250 µg and store at -20°C

Isolation of chondrocytes from 1 gr. bovine cartilage

DIGESTION SOLUTION FOR 1 gr OF CARTILAGE:
Solubilize one aliquot of Solution A in 10 ml DMEM and put it on ice. Immediately before use add one aliquot Solution B.

1. Transfer 1 gr. of cartilage shavings into a 150-mm petri dish containing DMEM+ 1% Pen-Strep + 1% Fungizone solution.

2. Mince the cartilage shavings into small chips (0.5 to 1 mm) using two sterile stainless steel scalpels, and transfer them into petri dish.

3. Add to the chips the **digestion solution + 1% Pen-Strep +1% Fungizone** and digest in incubator for 12-24 hrs at 37°C, 5% CO2.

4. Filter the collagenase/chips solution through a 20-µm nylon filter membrane: dissociated chondrocytes will pass through the filter. Divide the cell into two 50 ml tube and wash with 40 ml DMEM plus 10% FCS.

5. Centrifuge for 10 min at 250g r.t. to obtain a pellet containing chondrocytes. Aspirate the supernatant and wash the cells twice in DMEM plus 10% FCS.

6. Add 2 ml medium and suspend cells by pipetting up and down. Count cells. ~ 6-7 $x10^6$ condrocytes can be obtained from 1 gr of cartilage.

7. Plate the chondrocytes in cell culture plate for ~ 24 hrs to let primary chondrocytes completely attach. Then add an equivalent volume of medium, and refresh after 1 day.

***Note**: This protocol is meant to be a starting point; all isolation procedures require an individual optimization.COL G and COL H concentration, protease addition and digestion time can be experimentally adjusted.*