OSTEOBLASTS ISOLATION FROM CALVARIA OF MOUSE

Enzymes

COL G recombinant collagenase class I ACTIVITY ≥3.0 Units/mg (Pz Grassmann)

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 G in H_2O, do not exceed 30 U/ml final concentration.

2. Solution B: Reconstitute COL H in H_2O, do not exceed 300 U/ml final concentration.

3. Filter for sterility (0.22 µm) and make aliquots of Solution A and Solution B.

4. Annotate the Unit/ml value of the stock solutions and store at -20°C.

OSTEOBLASTS ISOLATION FROM 2 MICE CALVARIA

1. Anesthetize two mice (younger than 8 weeks) and euthanize them by cervical dislocation. Place the mice into the working platform ventral side down and clean the head using 70% ethanol. Then pick the calvaria.

2. Transfer the calvaria into a petri dish with PBS and remove the soft tissues. Remove the sutures and chop the remaining bone into small fragments of 1-2 mm^2.

3. DISSOCIATION SOLUTION: Prepare 12 ml of DMEM (containing Ca^{++} ≥ 2 mM) with a final concentration of 2.7 Units/ml COLG (Pz Grassmann from Solution A) + 11.8 Units/ml COL H (Pz Grassmann from Solution B).

4. Incubate the pieces of bone for 30 min with 4 ml DISSOCIATION SOLUTION in a shaking waterbath at 37°C.

5. Remove and refresh with 4 ml DISSOCIATION SOLUTION, for a second 30 min digestion.

6. Remove and add 4 ml of trypsin (5 mg/ml in PBS + EDTA 0.2 g/ml). Incubate for 30 min.

7. Replace again with 4 ml of DISSOCIATION SOLUTION for 30 minutes.

8. Rinse the bone fragments 3 times with DMEM+ 10% FBS and transfer the pieces of bone in a clean dish with 4 ml of complete DMEM with ascorbic acid (100 µg/ml).

9. Swirl occasionally to make sure that bone chips are evenly distributed over the bottom of the culture flask. Mouse bone cells will start migrating from the bone chips after 3-5 days.

9. After 11-15 days in culture incubate the cells with trypsin solution at 37°C for 10 min. Then remove the trypsin solution containing the cells using a small pipet and discard the bone pieces.

10. Plate the cells in a 24-well plate and culture for 7-10 days.

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.*

If you have any question please contact info@abielbiotech.com