

**Center for Skeletal Research**  
MGH Endocrine Unit

## Bone Cells Core

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<b>Primary Osteoblasts from Calvaria (Modified)</b>
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### Materials:

- Collagenase type I (Worthington -CLS1)
- Collagenase type II (Worthington -CLS2)
- HEPES solution (1M pH 7.4)
- Bovine Serum Albumin (powder or 10% stock solution)
- aMEM
- Complete medium (aMEM + 10% Fetal Bovine Serum + 1% Pen-Strep or Antimitotic/Antibiotic)

### For digestion:

A) Collagenase solution: 2.5 mg/ml collagenase1+collagenase 2 (ratio 1:3), 2,5mM CaCl<sub>2</sub> (solution should be made in ddH<sub>2</sub>O and filtered). Keep on ice

B) Medium: alpha MEM + 0.1 % BSA + 25 mM HEPES pH 7.4. medium should be at 37C

C) EDTA solution: 5mM EDTA in PBS + 0.1 % BSA (filtered)

D) Medium: Alpha MEM + 10%FBS+1%PS

### Procedure:

Keep pups (2-4 days old) on ice.

Quickly dip in EtOH, cut head, remove skin and isolate calvarial bones (parietal only)

Put the bone into serum free ice cold medium and keep them on ice.

At the end of all isolation prepare collagenase solution; Mix medium (B)+ collagenase (A) (2.5:1 ratio) to obtain final collagenase solution of 1mg/ml.

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Put calvarial bone into wells (if single calvaria =1 bone in 1 12well-plate, if combined 3-5 bones/1 6well) 12 or 6 well plate (depends on experiment) and add 1 ml (for 12-well) or 2 ml (for 6 wellplate) of collagenase solution (medium plus collagenase). Incubate at 37 C (plate warmer) on a shaker (~ 90 rpm/min = setting on 5)

**Digestion 1= 15 min collagenase solution.**

**Digestion 2= 20 min collagenase solution.**

Digestions 1 +2 are usually discarded (fibroblasts population?)

**Digestion 3-4 and 6 = 15 min collagenase.** Collect medium and add to a 15 ml tube containing 5 ml of complete medium a-MEM+10%FBS+PS (the FBS will inactivate the collagenase). Keep on ice. Wash the calvaria with 1 ml of collagenase solution and collect the wash in the same tube.

Collect together all 4 digestions. Spin, aspirate medium, resuspend in fresh and plate (Osteoblast enriched population)

**Digestion 5= 15 min EDTA.** Wash once with EDTA solution. Collect cells

**For osteocytes enriched population:**

**Digestion 5, 7= 15 min EDTA digestion.** No wash needed. Just collect cells.

**Digestion 8= 15 min collagenase.** Same as for digestion 3. Collect together with digestion 7. Spin and plate (Osteocyte enriched population)