



## Human Calvarial Osteoblasts (HCO)

Catalog Number: 4600

### Cell Specification

Bone is a dynamic tissue, being continuously remodeled by the coordinated actions of osteoclasts and osteoblast lineage. Osteoblasts, the bone-forming cells, are derived originally from pluripotent mesenchymal stem cells. They synthesize and secrete organic extracellular matrix, osteoid, which is composed primarily of type I collagen. Osteoid is calcified by osteoblasts and during this process the cells become encased in lacunae within the calcified material and become osteocytes. Osteoblasts express protease-activated receptor-1 and vesicular endothelial cell growth factor [1]. Studies show that Leukemia inhibitory factor can bind to the osteoblast cell surface and induce bone formation both *in vitro* and *in vivo* [2]. The balance between osteoblast recruitment, proliferation, differentiation and apoptosis in sutures between cranial bones is essential for calvarial bone formation [3].

HCO from ScienCell Research Laboratories are isolated from human calvariae. HCO are cryopreserved at primary cultures and delivered frozen. Each vial contains  $>5 \times 10^5$  cells in 1 ml volume. HCO are characterized by the cytochemically detection of AP and mineral deposition. HCO are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HCO are guaranteed for 15 population doublings at the conditions provided by ScienCell Research Laboratories.

### Recommended Medium

It is recommended to use Osteoblast Medium (OsM, Cat. No. 4601) for the culturing of HCO *in vitro*.

### Product Use

HCO are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

### Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture needed for experiments.

### Shipping

Dry ice.

### Reference

- [1] Steinbrech, D. S., Mehrara, B. J., Saadeh, P. B., Greenwald, J.A., Spector, J. A., Gittes, G. K. and Longaker, M. T. (2000) VEGF expression in an osteoblast-like cell line is regulated by a hypoxia response mechanism. *Am. J. Physiol. Cell Physiol.* 278: C853-C860.
- [2] Dazai, S., Akita, S., Hirano, A., Rashid, M. A., Naito, S., Akino, K., Fujii, T. (2000) Leukemia inhibitory factor enhances bone formation in calvarial bone defect. *J. Craniofac. Surg.* 11(6):513-20.
- [3] Marie, P. J., Debais, F., Hay, E. (2002) Regulation of human cranial osteoblast phenotype by FGF-2, FGFR-2 and BMP-2 signaling. *Histol. Histopathol.* 17(3):877-85.

# Instruction for culturing cells

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Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

## Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask ( $2 \mu\text{g}/\text{cm}^2$ , T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15  $\mu\text{l}$  of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm<sup>2</sup> is recommended.  
*Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that osteoblasts are plated in poly-L-lysine coated culture vessels that promote osteoblast attachment.*
6. Replace the cap or cover of flask, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

## Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

### **Subculture:**

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated flasks ( $2 \mu\text{g}/\text{cm}^2$ ) one day before subculture.
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS to **room temperature**. We do not recommend warming the reagents and medium at  $37^\circ\text{C}$  waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at  $37^\circ\text{C}$  incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at  $37^\circ\text{C}$  for 1 minutes (no solution in the flask at this moment); at the end of trypsinisation, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

*Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.*

6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter Allegra 6R centrifuge or similar*) for 5 min; re-suspend cells in growth medium.
7. Count cells and plate cells in a new, poly-L-lysine coated flask with cell density as recommended.

*Caution: Handling human derived products is potentially biohazardous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].*

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).