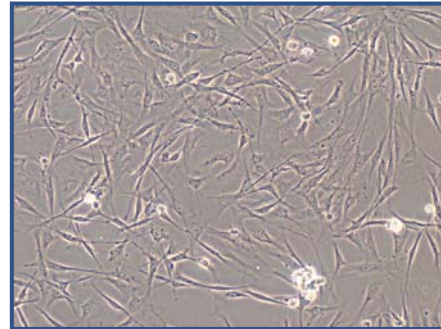


## Introduction

Primary bone osteoblast cells (BOBC) are isolated from the calvaria bone of neonatal Sprague-Dawley rats. In order to achieve high purification of cells, different collagenase digestion steps followed by repeated high-speed differential centrifugations were performed as described by Carpenter *et al*, 2005. Primary culture cell growth was allowed for six days prior to cryopreservation.



## Safety Statements

Health quality controls regularly performed on these rats including bacteriology, virus serology and parasitological controls, certify these animals to be free of pathogens. PCR testing on these cells gave negative results for presence of mycoplasma.

\* For research use only, not approved for application to humans, or for use in vitro diagnostic or clinical procedures.

## Product characteristics

**Organism:** *Rattus norvegicus* (rat)

**Strain:** Sprague-Dawley.

**Tissue:** Calvaria bone.

**Cell type:** Bone Osteoblast Cells.

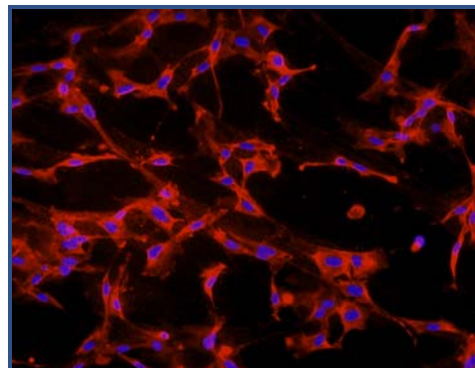
**Number of cells per vial:** > 0.5 x 10<sup>6</sup> viable bone osteoblast cells.

**N° Passages prior to cryopreservation:** 1

**Growth properties:** Adherent (plastic).

## Cell characterization

Isolated BOBC cells are cultured and phenotypically checked for proper morphology. In addition, cells are characterised by fluorescence immunostaining for the phenotype-specific marker alkaline phosphatase (shown in red). Nuclei are stained with DAPI (shown in blue).



### Handling instructions:

1. The recommended seeding density for attachment is approximately 7000 cells/cm<sup>2</sup> on plastic flasks/wells.
2. Use approximately 0.25 ml of culture medium per cm<sup>2</sup> of the plating surface and allow flasks/wells to equilibrate in a humidified 5% CO<sub>2</sub> incubator at 37°C for a minimum of 30 minutes.
3. Rapidly transfer the cryovial to thaw in a clean water bath at 37°C with gentle shaking. Monitor the contents of the vial, and when a small ice crystal remains remove the vial from the water bath. Wipe the cryovial with ethanol before opening.
4. Immediately mix the cell suspension using a micropipette avoiding the formation of bubbles and transfer it gently to the flasks previously prepared in step 2.
5. Help to distribute cells evenly by rocking the flasks gently. Loosen caps of flasks (unless vented caps are used) and return them to the incubator.
6. Change culture medium once cells are properly attached (no longer than 24 hours after seeding) and every second day thereafter. Pre-warm an appropriate amount of medium to 37°C before each medium change. Subculture cells when they are 70-90% confluent using Trypsin/EDTA solution.

\* Please, note that primary culture cells have a finite *in vitro* life-span.

### Culture medium

DMEM/high glucose (Sigma:D-5796) supplemented with 10 % fetal bovine serum (FBS; Sigma, F-7524), 10 µg/ml gentamycin (Sigma, G-1272) and 10 µg/ml penicillin-streptomycin (Sigma, A-5955).

### Storage:

Store in liquid nitrogen. For best results, use upon arrival.

### Note:

- ▶ *Dominion Pharmakine SL guarantees the performance of this product only when the recommended storage and protocols are followed.*
- ▶ *If this product does not arrive in good condition, please contact your distributor.*

