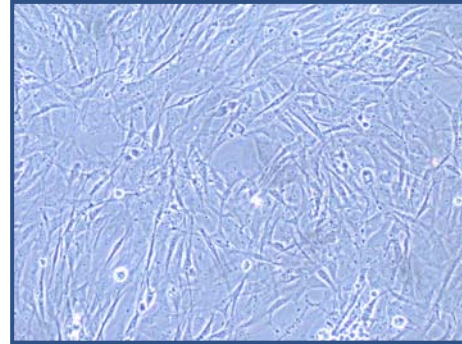


Introduction

Primary bone osteoblast cells (BOBC) are isolated from the calvaria bone of neonatal Balb-c mice. In order to achieve high purification of cells, different collagenase digestion steps followed by repeated high-speed differential centrifugations were performed by methods previously described (Carpenter *et al*, 2005.). Primary culture cell growth was allowed for 13 days prior to cryopreservation.



Safety Statements

Health quality controls regularly performed on these mice 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: *Mus musculus* (mouse)

Strain: Balb-c.

Tissue: Calvaria bone.

Cell type: Bone Osteoblast Cells.

Number of cells per vial: > 0.5 x 10⁶ viable bone osteoblast cells.

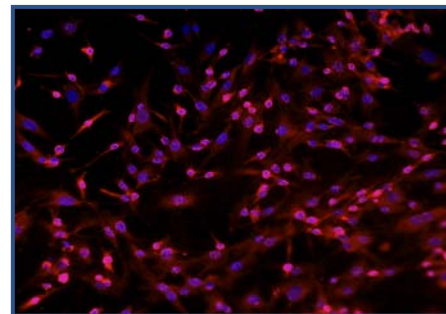
Doubling time prior cryopreservation: 5 days.

N^o Passages: 2 passages.

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/cm² on plastic flasks/wells.
2. Use approximately 0.25 ml of culture medium per cm² of the plating surface and allow flasks/wells to equilibrate in a humidified 5% CO₂ 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*

