Osteoblasts

Instruction Manual

Product Description

Human osteoblasts are a highly specialized cell type of mesenchymal origin involved in bone formation and remodeling. In vivo, they produce the osteoid, an extracellular matrix rich in collagen type I. During their natural maturation process into osteocytes, called osteogenesis, they become embedded in the bone matrix and stop proliferating. Our Human Osteoblasts (HOB) are produced at our cell culture facility and are available from knee and hip femoral tissue. Shortly after isolation, all Human Osteoblasts are cryopreserved at passage 2 (P2) using our proprietary, defined, animal-component free, and protein-free cryopreservation medium, Cryo-SFM. Each cryovial contains more than 500,000 viable cells after thawing. Proliferating cell cultures are made from cryopreserved cells that have been thawed and cultured for three days in our hands and shipped as growing cultures.

Quality Control

We perform rigid quality control tests for each lot of Human Osteoblasts. The cells are tested for cell morphology, adherence rate, and cell viability. Furthermore, histochemical tests for alkaline phosphatase and bone mineralization are carried out for each lot (see page 5). Growth performance is tested through multiple passages up to 10 population doublings (PD) under culture conditions lacking antibiotics or antimycotics.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1 and HTLV-2, the cells – like all products of human origin – should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

A detailed certificate of analysis (CoA) for each lot can be downloaded at: www.promocell.com/coa

Intended Use

Our Human Osteoblasts are for in vitro research use only and not for diagnostic or therapeutic procedures.

Warning

Although tested negative for HIV-1, HIV-2, HBV, HCV, HTLV-1 and HTLV-2, the cells – like all products of human origin – should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

Follow appropriate safety precautions!

After delivery, cryopreserved cells should be stored in liquid nitrogen or seeded directly (see page 2). Proliferating cells must be processed immediately (see page 3).

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Size</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Osteoblasts (HOB)</td>
<td>500,000 cryopreserved cells 500,000 proliferating cells</td>
<td>C-12720 C-12760</td>
</tr>
</tbody>
</table>
Protocol for Cryopreserved Cells

Straight after arrival, store the cryopreserved cells in liquid nitrogen, or seed them immediately.

**Note:** Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

*Use aseptic techniques and a laminar flow bench.*

1. **Prepare the medium**
   Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (37°C, 5% CO₂) for 30 minutes.

2. **Thaw the cells**
   Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.

3. **Disinfect the vial and seed the cells**
   Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a cell culture vessel containing the prewarmed medium from step 1.

4. **Incubate the cells**
   Place the vessel in an incubator (37°C, 5% CO₂) for cell attachment. Replace the medium after 16–24 hours and every two to three days thereafter. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70–90% confluency.
Start immediately after delivery.
Use aseptic techniques and a laminar flow bench.

Protocol for Proliferating Cells

1. Incubate the cells

Unpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% CO₂) for 3 hours to allow the cells to recover from transportation.

2. Replace the transport medium

Carefully open the vessel, rinse the inner side of the cap with 70% ethanol, and let air dry. Aspirate the transport medium from the vessel. Add 10 ml of the appropriate PromoCell Cell Growth Medium.

3. Check and incubate the cells

Check the cell density. Open the cap half a turn and place the vessel in an incubator (37°C, 5% CO₂). Change the medium every two to three days. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached >70% confluency.
Subcultivation Protocol

Prepare the reagents and wash the cells

Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm² of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.

Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm² of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.

Neutralize the trypsin and harvest the cells

Add 100 µl Trypsin Neutralization Solution per cm² of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.

Incubate the cells

Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Cell Growth Medium (step 2), and resuspend the cells by carefully pipetting up and down. Plate the cells according to the recommended seeding density in new cell culture vessels containing prewarmed PromoCell Growth Medium. Place the vessels in an incubator (37°C, 5% CO₂) and change the media every two to three days.
## Specifications

<table>
<thead>
<tr>
<th>Product</th>
<th>Recommended Culture Media*</th>
<th>Plating Density</th>
<th>Passage after Thawing</th>
<th>Marker</th>
<th>Population Doublings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Osteoblasts (HOB)</td>
<td>C-27001 C-27020</td>
<td>10,000 – 20,000 cells per cm²</td>
<td>P2</td>
<td>Alkaline phosphatase* Mineralization tested</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>

*The catalog numbers in this table are for media in ready-to-use packaging.

## Related Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Size</th>
<th>Catalog Number</th>
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</thead>
<tbody>
<tr>
<td>Osteoblast Growth Medium (Ready-to-use)</td>
<td>500 ml</td>
<td>C-27001</td>
</tr>
<tr>
<td>Osteoblast Basal Medium</td>
<td>500 ml</td>
<td>C-27010</td>
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<tr>
<td>Osteoblast Basal Medium, phenol red-free</td>
<td>500 ml</td>
<td>C-27015</td>
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<tr>
<td>Osteoblast Growth Medium SupplementMix</td>
<td>for 500 ml</td>
<td>C-39615</td>
</tr>
<tr>
<td>Osteoblast Mineralization Medium (Ready-to-use)</td>
<td>100 ml</td>
<td>C-27020</td>
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<tr>
<td>DetachKit</td>
<td>30 ml 125 ml 250 ml</td>
<td>C-41200 C-41210 C-41220</td>
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<tr>
<td>Cryo-SFM</td>
<td>30 ml 125 ml</td>
<td>C-29910 C-29912</td>
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<tr>
<td>HOB Pellet</td>
<td>1 million cells per pellet</td>
<td>C-14071</td>
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