Osteoblasts

PromoCell®

Instruction manual

Product	Size	Catalog number
Human Osteoblasts (HOB)	500,000 cryopreserved cells 500,000 proliferating cells	C-12720 C-12760

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 our Human Osteoblasts are cryopreserved using our proprietary, animal-component free, and protein-free cryopreservation medium (for details, please refer to the Certificate of Analysis).

Each cryovial contains more than 500,000 viable cells after thawing. Thawing and seeding results in passage 2. 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, and microbial contaminants (fungi, bacteria, and mycoplasma).

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).

Protocol for cryopreserved cells

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

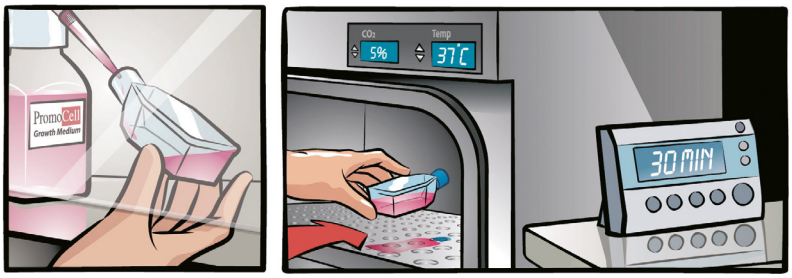
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium

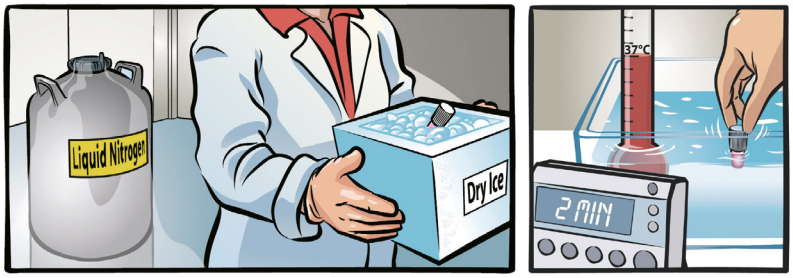
Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (37°C, 5% CO_2) for 30 minutes.



2

Thaw the cells

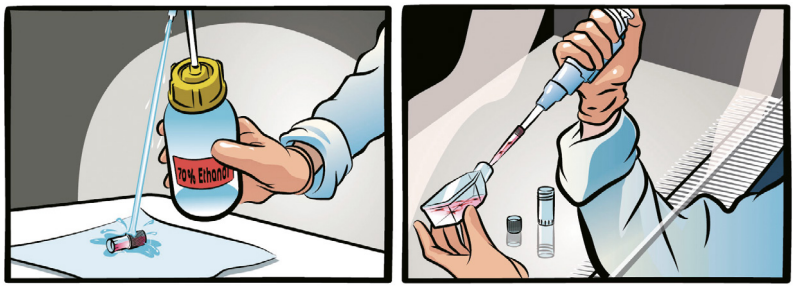
Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for 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 pre-warmed medium from step 1.



4

Incubate the cells

Place the vessel in an incubator (37°C, 5% CO_2) 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.



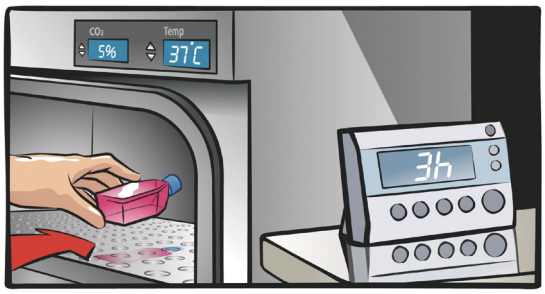
Protocol for proliferating cells

Start immediately after delivery.
Use aseptic techniques and a laminar flow bench.

1

Incubate the cells

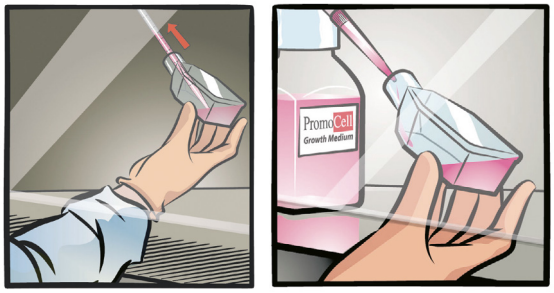
Unpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from transportation.



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% CO_2). 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.



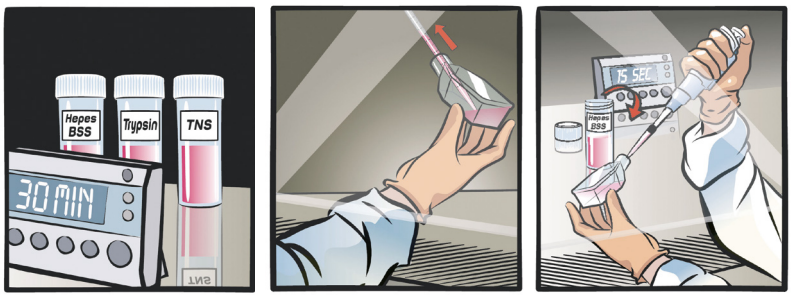
Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

Prepare the reagents and wash the cells

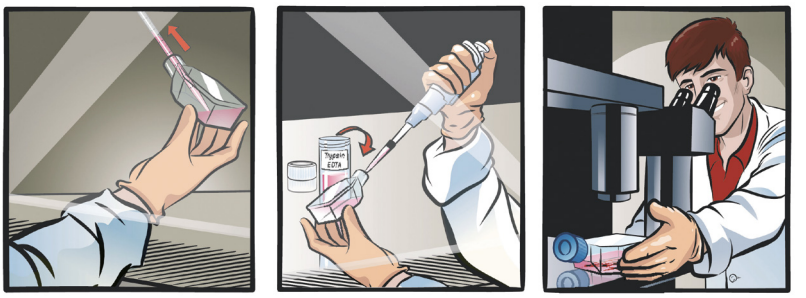
Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm^2 of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



2

Detach the cells

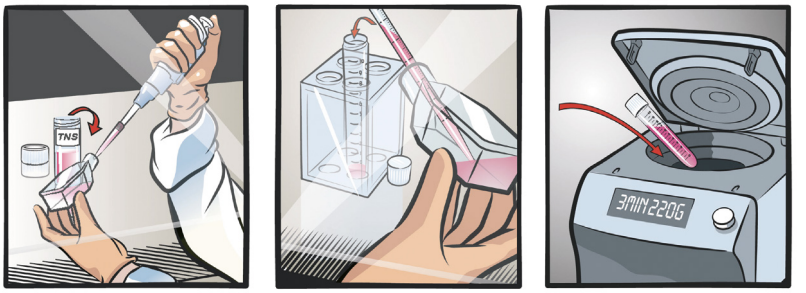
Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm^2 of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.



3

Neutralize the trypsin and harvest the cells

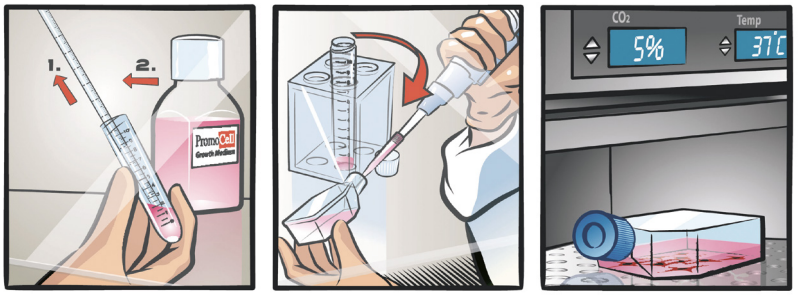
Add 100 µl Trypsin Neutralization Solution per cm^2 of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



4

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% CO_2) and change the media every two to three days.



Specifications

Product	Recommended culture media*	Plating density	Passage after thawing	Marker	Population doublings
Human Osteoblasts (HOB)	C-27001 C-27020	10,000 – 20,000 cells per cm^2	P2	Alkaline phosphatase+ Mineralization tested	> 10

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

Related products

Product	Size	Catalog number
Osteoblast Growth Medium (Ready-to-use)	500 ml	C-27001
Osteoblast Basal Medium	500 ml	C-27010
Osteoblast Basal Medium, phenol red-free	500 ml	C-27015
Osteoblast Growth Medium SupplementMix	for 500 ml	C-39615
Osteoblast Mineralization Medium (Ready-to-use)	100 ml	C-27020
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM Plus	30 ml 125 ml	C-29920 C-29922
HOB Pellet	1 million cells per pellet	C-14071

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