Pericytes

PromoCell

Instruction Manual

Product	Size	Catalog Number
Human Pericytes from Placenta (hPC-PL)	500,000 cryopreserved cells 500,000 proliferating cells	C-12980 C-12981

Product Description

Pericytes are multipotent mesenchymal-like cells found in association with small blood vessel walls. They are important for angiogenesis, the structural integrity of the microvasculature, and blood flow regulation. However, they can also develop into malignant tumors called hemangiopericytomas.

To date, these cells have demonstrated the ability to differentiate into adipocytes, osteoblasts, fibroblasts, smooth muscle cells, and phagocytes (macrophages).

PromoCell offers placenta-derived Pericytes produced at PromoCell's cell culture facility from normal human tissues. Shortly after isolation, all Pericytes are cryopreserved at passage 2 (P2) using PromoCell's proprietary, serum-free freezing medium, Cryo-SFM. Each cryo vial 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 at PromoCell.

Quality Control

Rigid quality control tests are performed for each lot of PromoCell Pericytes.

The cells are tested for morphology, adherence rate, and viability. Furthermore, they are characterized by flow cytometric analysis for a comprehensive panel of markers, e.g. CD31, CD34, CD105, and CD146.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, and microbial contaminants (fungi, bacteria, and mycoplasma).

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

Intended Use

PromoCell Pericytes are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

Warning

Although tested negative for HIV-1, HIV-2, HBV, and HCV, 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 have to be processed immediately (see page 3).

Use aseptic techniques and a laminar flow bench.

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.

1. Prepare the medium

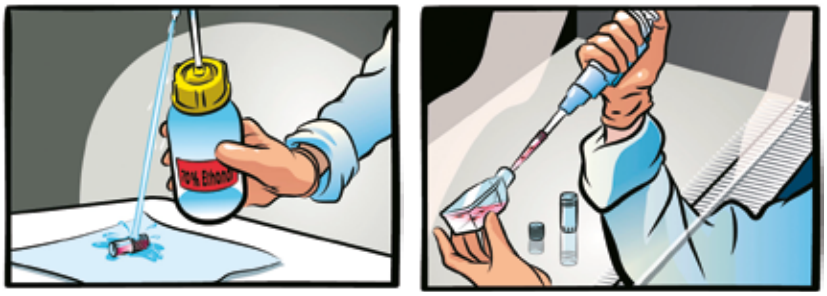
Calculate the needed culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (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 into a water bath (37°C) just up to 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% 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.

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% CO_2) for 3 hours to allow the cells to recover from the 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 or three days. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached >70% confluency.

Use aseptic techniques and a laminar flow bench.

Subcultivation Protocol

1. Prepare the reagents and wash the cells

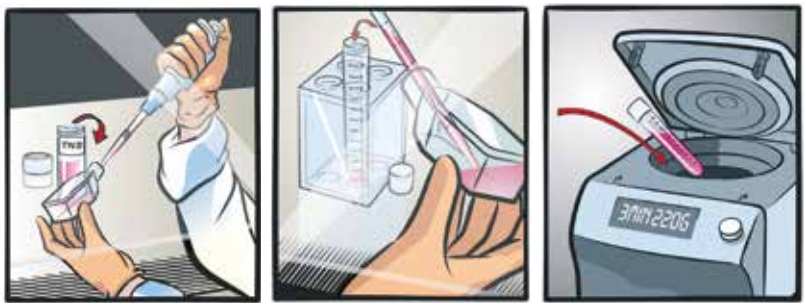
Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm² of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.

2. Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per 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.

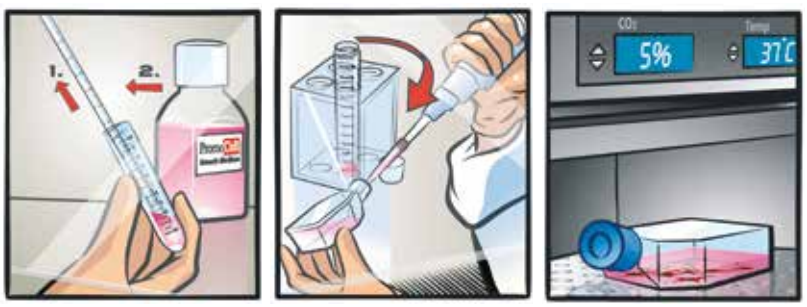
3. Neutralize the trypsin and harvest the cells

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.



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 Cell 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
Human Pericytes from Placenta (hPC-PL)	C-28040	3,000 – 4,000 cells per cm²	P2

Related Products

Product	Size	Catalog Number
Pericyte Growth Medium (Ready-to-use)	500 ml	C-28040
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
hPC-PL Pellet	1 million cells per pellet	C-14095
PromoFectin Cell Transfection Reagent	0.1 ml 0.5 ml 1 ml	PK-CT-2000-10 PK-CT-2000-50 PK-CT-2000-100
Recombinant Human Platelet Derived Growth Factor BB (E. coli-derived)	10 µg	C-63022
Recombinant Human Vascular Endothelial Cell Growth Factor 165 (E. coli-derived)	10 µg	C-64420
Recombinant Human Transforming Growth Factor beta-1 (113 aa, E. coli-derived)	10 µg	C-63500
Recombinant Human Angiopoietin 1 (HeLa cell-derived)	10 µg	C-60014

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