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).

We offer placenta-derived Pericytes produced at our cell culture facility from normal human tissues. Shortly after isolation, all pericytes 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 or three days in our hands and shipped as growing cultures.

Quality Control

We perform rigid quality control tests for each lot of Pericytes. The cells are tested for cell morphology, proliferation potential, adherence rate, and viability. Furthermore, they are characterized by flow cytometric analysis of a comprehensive panel of markers, namely 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

Our 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, 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

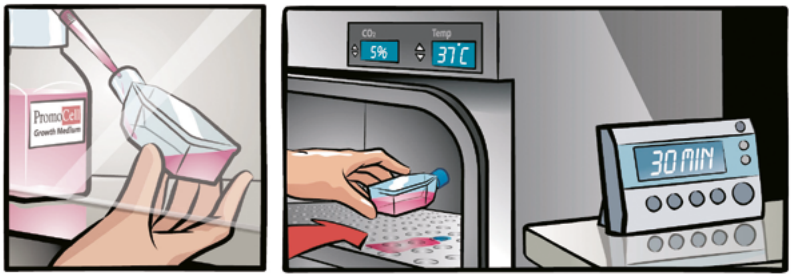
Straight after arrival, store the cryopreserved cells in liquid nitrogen or seed them immediately.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium and the culture vessel

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

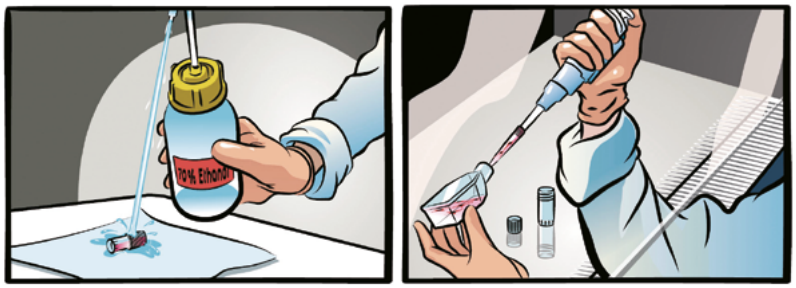
Thaw the cells

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.

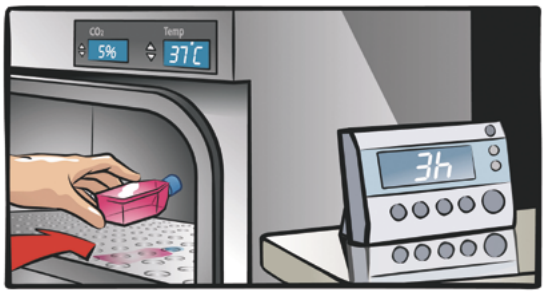
Protocol for Proliferating Cells

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

1

Incubate the cells

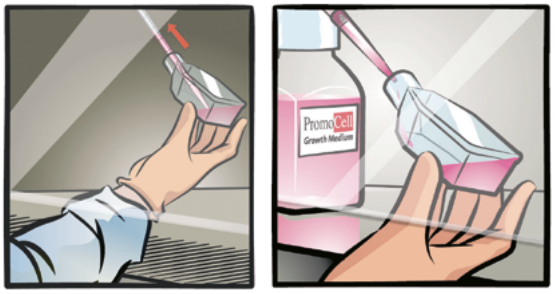
Unpack the culture vessel, do not open the lid, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from transportation.



2

Replace the transport medium

Carefully open the vessel, rinse the inner side of the lid 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 lid 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.

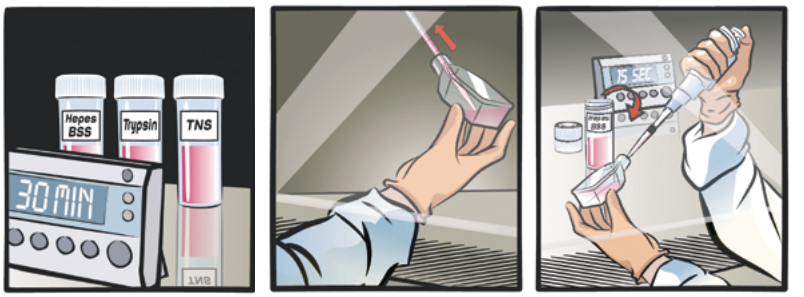
Subcultivation Protocol

Use aseptic techniques and a laminar flow bench.

1

Prepare the reagents and wash the cells

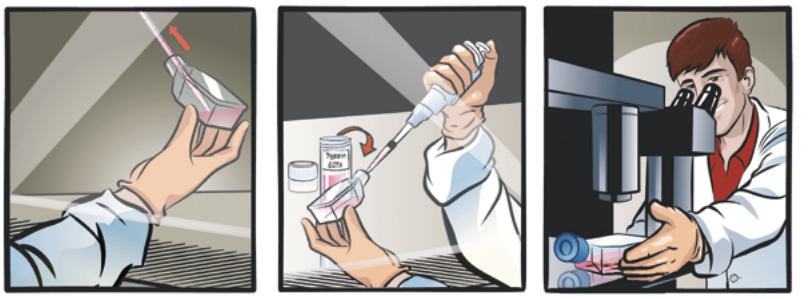
Place the Accutase-Solution (C-41310) at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS or PBS per cm^2 of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



2

Detach the cells

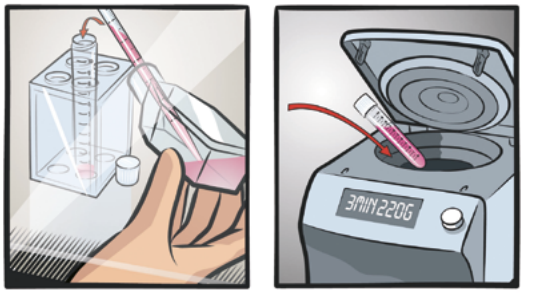
Carefully aspirate the Hepes BSS or PBS from the culture vessel. Add 100 µl Accutase-Solution per cm^2 of vessel surface and incubate for two to four minutes at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.



3

Harvest the cells

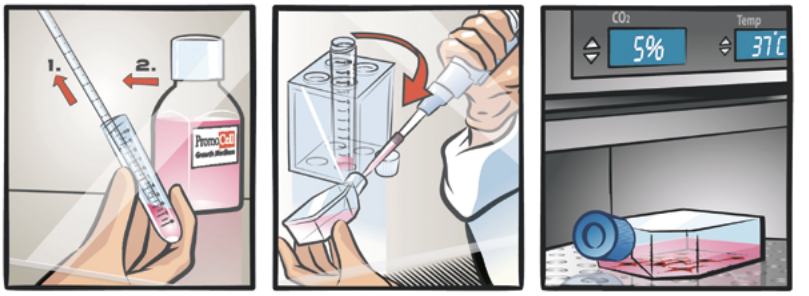
Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



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 PromoCell Cell Growth Medium prewarmed to 37°C. 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-28041	3,000 – 4,000 cells per cm^2	P2

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

Related Products

Product	Size	Catalog Number
Pericyte Growth Medium 2 (Ready-to-use)	500 ml	C-28041
Accutase-Solution, primary human cell culture tested	100 ml	C-41310
HEPES Buffered Saline Solution	125 ml 250 m	C-40010 C-40020
hPC-PL Pellet	1 million cells per pellet	C-14095
Cryo-SFM	30 ml 125 ml	C-29910 C-29912

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