Smooth Muscle Cells

PromoCell®

Instruction Manual

Product	Size	Catalog Number
Human Aortic Smooth Muscle Cells (HAoSMC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12533 C-12532
Human Coronary Artery Smooth Mus-cle Cells (HCASMC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12511 C-12512
Human Pulmonary Artery Smooth Mus-cle Cells (HPASMC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12521 C-12522
Human Umbilical Artery Smooth Mus-cle Cells (HUASMC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12500 C-12550
Human Tracheal Smooth Muscle Cells (HTSMC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12565 C-12566
Human Bronchial Smooth Muscle Cells (HBSMC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12561 C-12562
Human Uterine Smooth Muscle Cells (HUtSMC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12575 C-12576

Product Description

Smooth muscle tissue is found in the *tunica media* layer of large and small blood vessels and in the walls of hollow organs like the bladder and the uterus. Smooth Muscle Cells (SMC) possess all the same types of filaments, but depending on the tissue of origin, differ significantly in mechanical and physiological properties. Therefore, we offer a range of human smooth muscle cells produced at our cell culture facility from normal human tissue of different origins. The cells are isolated from the aorta, the coronary artery, the pulmonary artery, the umbilical artery, the trachea, the bronchi, and the uterus.

Shortly after isolation, all Human Smooth Muscle Cells 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 at PromoCell.

Quality Control

We perform rigid quality control tests for each lot of Human Smooth Muscle Cells.
The cells are tested for cell morphology, adherence rate, and cell viability. Flow cytometric analyses for cell-type specific markers, e.g. smooth muscle α-actin (see page 5), are carried out for each lot. Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics or antimycotics.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2 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 Human Smooth Muscle Cells 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.

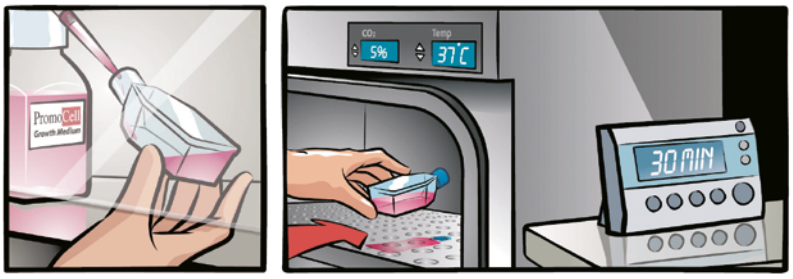
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium and the culture vessel

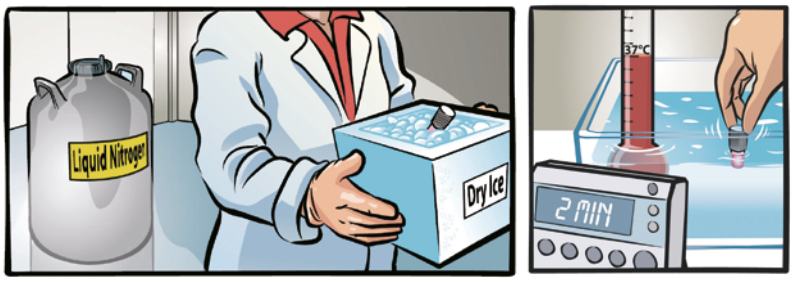
Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an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



2

Thaw the cells

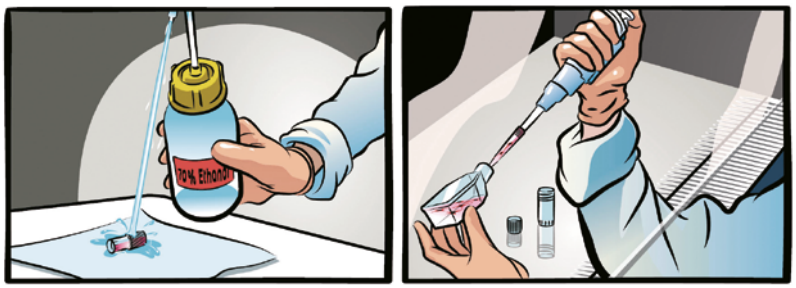
Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for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

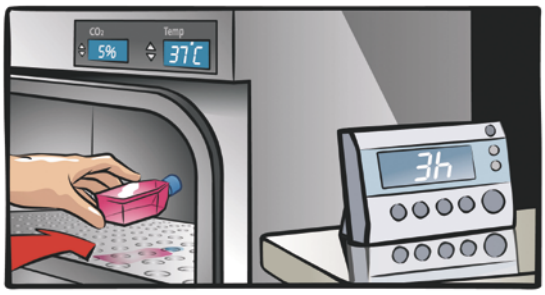
Protocol for Proliferating Cells

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

1

Incubate the cells

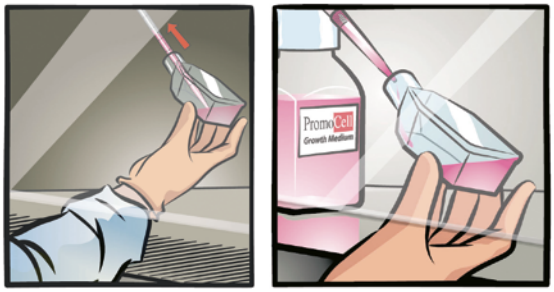
UUnpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from the transportation.



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 to three days. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached >70% confluency.

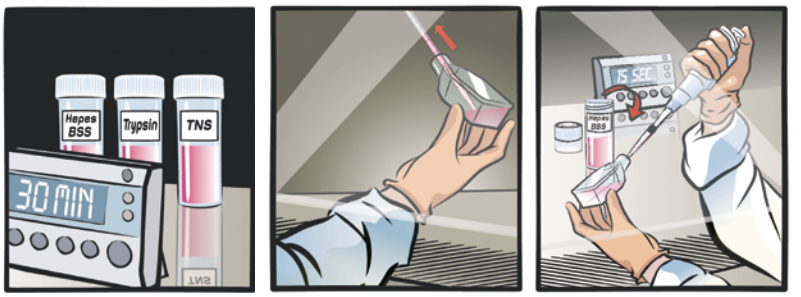
Subcultivation Protocol

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.

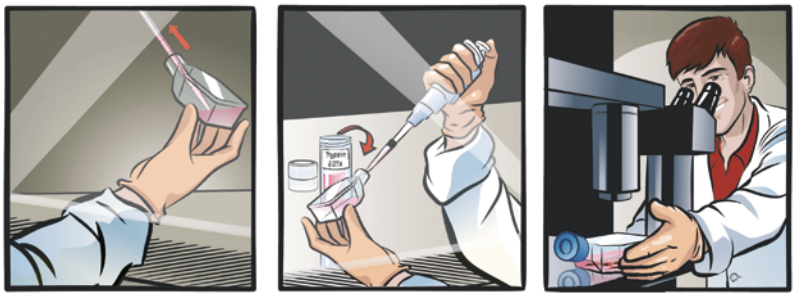


2

Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm^2 of vessel surface.

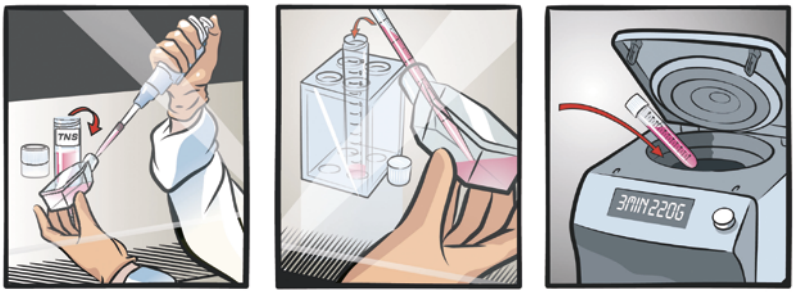
Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.



3

Neutralize the trypsin and harvest the cells

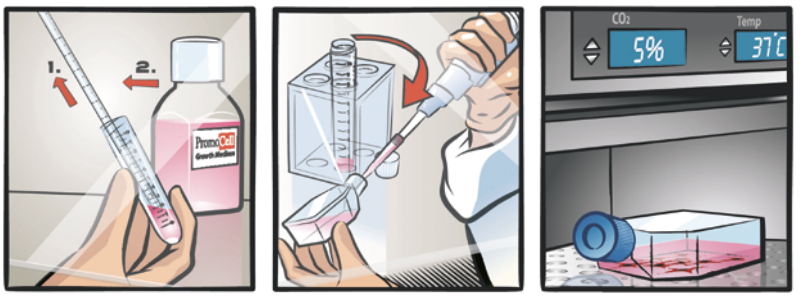
Add 100 µl Trypsin Neutralization Solution per cm^2 of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



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 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	Marker	Population Doublings
Human Aortic Smooth Muscle Cells (HAoSMC)	C-22062	7,500 – 10,000 cells per cm²	P2	Smooth muscle α-actin⁺	> 15
Human Coronary Ar-tery Smooth Muscle Cells (HCASMC)	C-22062	7,500 – 10,000 cells per cm²	P2	Smooth muscle α-actin⁺	> 15
Human Pulmonary Ar-tery Smooth Muscle Cells (HPASMC)	C-22062	7,500 – 10,000 cells per cm²	P2	Smooth muscle α-actin⁺	> 15
Human Umbilical Ar-tery Smooth Muscle Cells (HUASMC)	C-22062	7,500 – 10,000 cells per cm²	P2	Smooth muscle α-actin⁺	> 15
Human Tracheal Smooth Muscle Cells (HTSMC)	C-22062	7,500 – 10,000 cells per cm²	P2	Smooth muscle α-actin⁺	> 15
Human Bronchial Smooth Muscle Cells (HBSMC)	C-22062	7,500 – 10,000 cells per cm²	P2	Smooth muscle α-actin⁺	> 15
Human Uterine Smooth Muscle Cells (HUtSMC)	C-22062	7,500 – 10,000 cells per cm²	P2	Smooth muscle α-actin⁺	> 15

Related Products

Product	Size	Catalog Number
Smooth Muscle Cell Growth Medium 2 (Ready-to-use)	500 ml	C-22062
Smooth Muscle Cell Growth Medium 2 Kit	500 ml	C-22162
Smooth Muscle Cell Basal Medium 2	500 ml	C-22262
Smooth Muscle Cell Basal Medium 2, phenol red-free	500 ml	C-22267
Smooth Muscle Cell Growth Medium 2 SupplementMix	for 500 ml	C-39267
Smooth Muscle Cell Growth Medium 2 SupplementPack	for 500 ml	C-39262
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
HAoSMC Pellet	1 million cells per pellet	C-14053
HCASMC Pellet	1 million cells per pellet	C-14052
HPASMC Pellet	1 million cells per pellet	C-14051
HUASMC Pellet	1 million cells per pellet	C-14050
HTSMC Pellet	1 million cells per pellet	C-14056
HBSMC Pellet	1 million cells per pellet	C-14055
HUtSMC Pellet	1 million cells per pellet	C-14059
Human Pericytes from Placenta (hPC-PL)	500,000 cryopreserved cells 500,000 proliferating cells	C-12980 C-12981

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