Endothelial cells (Microvascular)

PromoCell®

Instruction manual

Product	Size	Catalog number
Human Dermal Microvascular Endothelial Cells (HDMEC) juvenile foreskin	500,000 cryopreserved cells 500,000 proliferating cells	C-12210 C-12260
Human Dermal Microvascular Endothelial Cells (HDMEC) adult donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12212 C-12262
Human Dermal Microvascular Endothelial Cells (HDMEC) pre-screened	500,000 cryopreserved cells 500,000 proliferating cells	C-12215 C-12265
Human Dermal Blood Endothelial Cells (HDBEC) juvenile foreskin	500,000 cryopreserved cells 500,000 proliferating cells	C-12211 C-12214
Human Dermal Blood Endothelial Cells (HDBEC) adult donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12225 C-12226
Human Dermal Lymphatic Endothelial Cells (HDLEC) juvenile foreskin	500,000 cryopreserved cells 500,000 proliferating cells	C-12216 C-12218
Human Dermal Lymphatic Endothelial Cells (HDLEC) adult donor	500,000 cryopreserved cells 500,000 proliferating cells	C-12217 C-12219
Human Cardiac Microvascular Endothelial Cells (HCMEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12285 C-12286
Human Pulmonary Microvascular Endothelial Cells (HPMEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12281 C-12282
Human Uterine Microvascular Endothelial Cells (HUtMEC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12295 C-12296

Product description

The walls of capillaries are composed of a single layer of microvascular endothelial cells. These cells differ in morphology and other properties depending on the tissues the capillaries supply. Therefore, we offer a range of Microvascular Endothelial Cells produced at our cell culture facility from normal human tissues of different origins. Shortly after isolation, Human Cardiac Microvascular Endothelial Cells (HCMEC) are cryopreserved at passage 3 (P3) and all other Microvascular Endothelial 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 500,000 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 Microvascular Endothelial Cells.
The cells are tested for cell morphology and cell-type specific markers, e.g. CD31 and Podoplanin, (see page 5) using flow cytometric analyses.

Growth performance is tested through multiple passages up to 10 or 15 (see page 5) population doublings (PD) without antibiotics or antimycotics.

In addition, all cells have been tested for the absence of HIV-1, 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 Microvascular Endothelial Cells are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

Warning

Although tested negative for HIV-1, 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, start immediately with the protocol for cryopreserved cells (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.

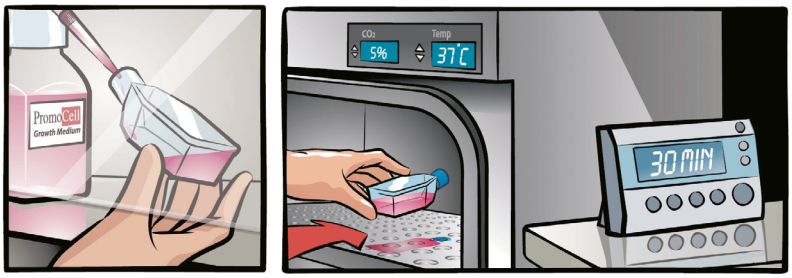
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium

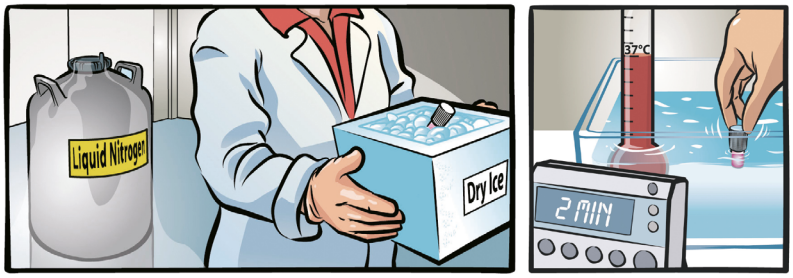
Calculate the required culture surface area according to the plating density (see page 5). Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (37°C, 5% CO_2) for 30 minutes.



2

Thaw the cells

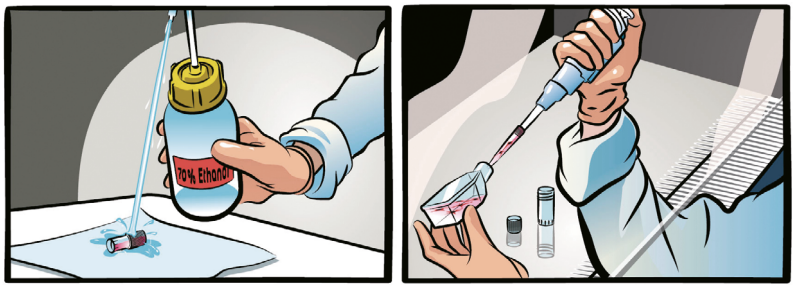
Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for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

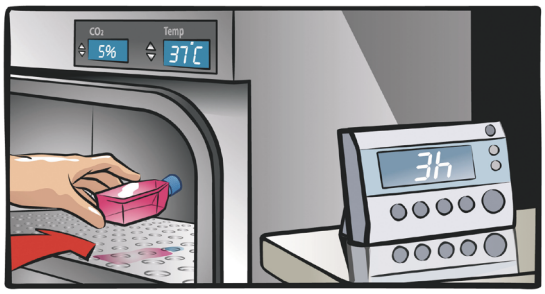
Protocol for proliferating cells

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

1

Incubate the cells

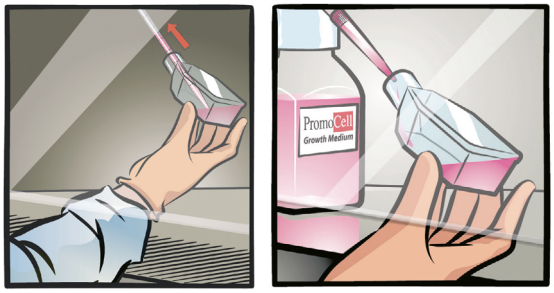
Unpack the culture vessel, do not open the cap, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from transportation.



2

Replace the transport medium

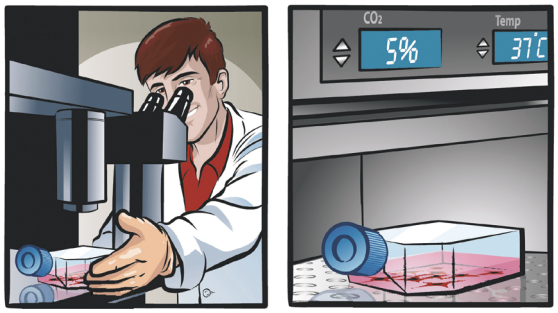
Carefully open the vessel, rinse the inner side of the lid with 70% ethanol, and let air dry. Aspirate the transport medium from the vessel. Add 10 ml of the appropriate PromoCell Cell Growth Medium.



3

Check and incubate the cells

Check the cell density. Open the 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 70–90% confluency.



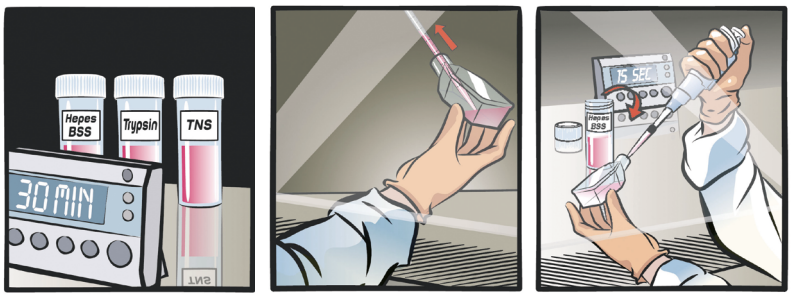
Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

Prepare the reagents and wash the cells

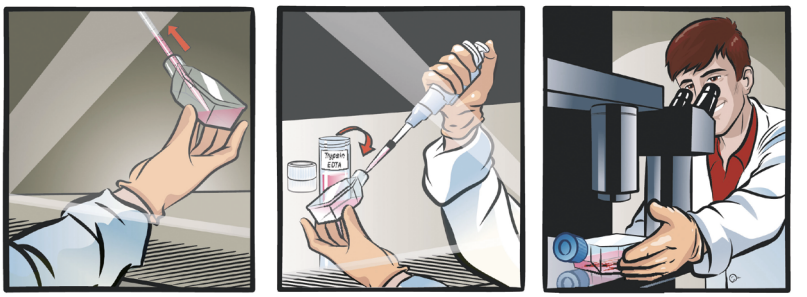
Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm² of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



2

Detach the cells

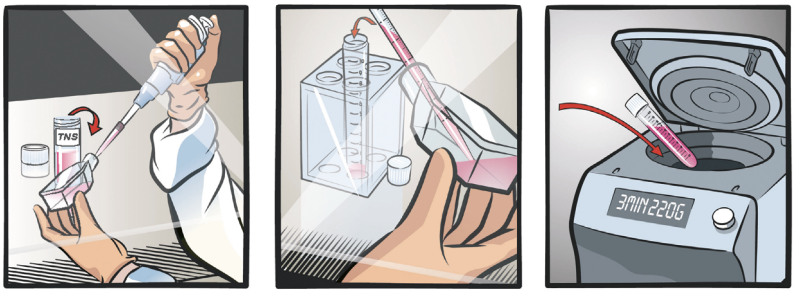
Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm² of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel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



3

Neutralize the trypsin and harvest the cells

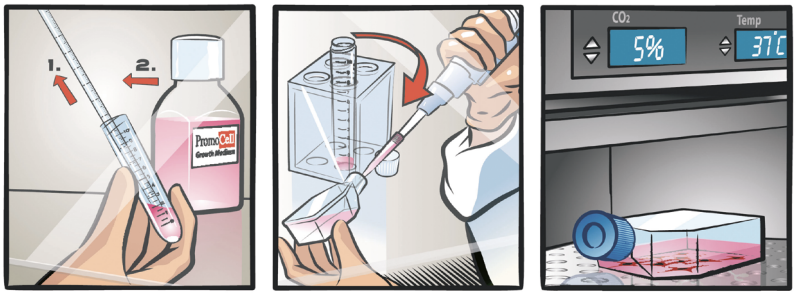
Add 100 µl Trypsin Neutralization Solution per cm² of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



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	Marker	Population doublings
Human Dermal Microvascular Endothelial Cells (HDMEC) juvenile foreskin	C-22020 C-22022	10,000 – 20,000 cells per cm^2	P2	$CD31^+$ Dil-Ac-LDL $uptake^+$	> 15
Human Dermal Microvascular Endothelial Cells (HDMEC) adult donor	C-22020 C-22022	10,000 – 20,000 cells per cm^2	P2	$CD31^+$ Dil-Ac-LDL $uptake^+$	> 15
Human Dermal Microvascular Endothelial Cells (HDMEC) pre-screened	C-22020 C-22022	10,000 – 20,000 cells per cm^2	P2	$CD31^+$ Dil-Ac-LDL $uptake^+$ VEGF $response^+$	> 15
Human Dermal Blood Endothelial Cells (HDBEC) juvenile foreskin	C-22020	10,000 – 20,000 cells per cm^2	P2	$Podoplanin^-$ $CD31^+$	> 15
Human Dermal Blood Endothelial Cells (HDBEC) adult donor	C-22020	10,000 – 20,000 cells per cm^2	P2	$Podoplanin^-$ $CD31^+$	> 15
Human Dermal Lymphatic Endothelial Cells (HDLEC) juvenile foreskin	C-22022	10,000 – 20,000 cells per cm^2	P2	$Podoplanin^+$ $CD31^+$	> 15
Human Dermal Lymphatic Endothelial Cells (HDLEC) adult donor	C-22022	10,000 – 20,000 cells per cm^2	P2	$Podoplanin^+$ $CD31^+$	> 15
Human Cardiac Microvascular Endothelial Cells (HCMEC)	C-22020 C-22022	10,000 – 20,000 cells per cm^2	P3	$CD31^+$ Dil-Ac-LDL $uptake^+$	> 10
Human Pulmonary Microvascular Endothelial Cells (HPMEC)	C-22020 C-22022	10,000 – 20,000 cells per cm^2	P2	$CD31^+$ Dil-Ac-LDL $uptake^+$	> 15
Human Uterine Microvascular Endothelial Cells (HUtMEC)	C-22020 C-22022	10,000 – 20,000 cells per cm^2	P2	$CD31^+$ Dil-Ac-LDL $uptake^+$	> 15

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

Related products

Product	Size	Catalog number
Endothelial Cell Growth Medium MV (Ready-to-use)	500 ml	C-22020
Endothelial Cell Growth Medium MV Kit	500 ml	C-22120
Endothelial Cell Basal Medium MV	500 ml	C-22220
Endothelial Cell Basal Medium MV, phenol red-free	500 ml	C-22225
Endothelial Cell Growth Medium MV SupplementMix	for 500 ml	C-39225
Endothelial Cell Growth Medium MV SupplementPack	for 500 ml	C-39220
Endothelial Cell Growth Medium MV 2 (Ready-to-use)	500 ml	C-22022
Endothelial Cell Growth Medium MV 2 Kit	500 ml	C-22121

Product	Size	Catalog number
Endothelial Cell Basal Medium MV 2	500 ml	C-22221
Endothelial Cell Basal Medium MV 2, phenol red-free	500 ml	C-22226
Endothelial Cell Growth Medium MV 2 SupplementMix	for 500 ml	C-39226
Endothelial Cell Growth Medium MV 2 SupplementPack	for 500 ml	C-39221
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
HDMEC Pellet	> 1 million cells per pellet	C-14015
HDMEC adult Pellet	> 1 million cells per pellet	C-14016
HDMEC pre-screened Pellet	> 1 million cells per pellet	C-14017
HDBEC Pellet	> 1 million cells per pellet	C-14018
HDBEC adult Pellet	> 1 million cells per pellet	C-14019
HDLEC Pellet	> 1 million cells per pellet	C-14020
HDLEC adult Pellet	> 1 million cells per pellet	C-14021
HCMEC Pellet	> 1 million cells per pellet	C-14029
HPMEC Pellet	> 1 million cells per pellet	C-14027
HUtMEC Pellet	> 1 million cells per pellet	C-14028

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