

# Endothelial Cells (Large Vessels)

PromoCell

## Instruction Manual

Product	Size	Catalog Number
Human Umbilical Vein Endothelial Cells (HUVEC) single donor	500,000 cryopreserved cells	C-12200
	500,000 proliferating cells	C-12250
Human Umbilical Vein Endothelial Cells (HUVEC) pooled	500,000 cryopreserved cells	C-12203
	500,000 proliferating cells	C-12253
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, single donor	500,000 cryopreserved cells	C-12206
	500,000 proliferating cells	C-12207
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, pooled	500,000 cryopreserved cells	C-12208
	500,000 proliferating cells	C-12209
Human Umbilical Vein Endothelial Cells (HUVEC) pre-screened	500,000 cryopreserved cells	C-12205
	500,000 proliferating cells	C-12255
Human Umbilical Artery Endothelial Cells (HUAEC)	500,000 cryopreserved cells	C-12202
	500,000 proliferating cells	C-12252
Human Aortic Endothelial Cells (HAoEC)	500,000 cryopreserved cells	C-12271
	500,000 proliferating cells	C-12272
Human Coronary Artery Endothelial Cells (HCAEC)	500,000 cryopreserved cells	C-12221
	500,000 proliferating cells	C-12222
Human Pulmonary Artery Endothelial Cells (HPAEC)	500,000 cryopreserved cells	C-12241
	500,000 proliferating cells	C-12242
Human Saphenous Vein Endothelial Cells (HSaVEC)	500,000 cryopreserved cells	C-12231
	500,000 proliferating cells	C-12232

### Product Description

PromoCell endothelial cells from large vessels are available from different locations, e.g. umbilical vein, umbilical artery, aorta, coronary artery, pulmonary artery, and saphenous vein.

In addition, PromoCell offers HUVEC isolated in standard Endothelial Cell Growth Medium and also in Endothelial Cell Growth Medium 2. The cells are supplied either from single donors\* or from pooled donors (from up to four different umbilical cords). Furthermore, HUVEC are available which are pre-screened for VEGF (vascular endothelial growth factor) response.

Shortly after isolation, PromoCell Human Endothelial Cells from large vessels are cryopreserved at passage 1 or passage 2 (see page 5) using PromoCell's proprietary, serum-free freezing 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

Rigid quality control tests are performed for each lot of Endothelial Cells from large vessels.

They are tested for cell morphology, adherence rate, and cell viability. Flow cytometric analyses for cell-type specific markers, e.g. von Willebrand Factor (vWF) and CD31 (see page 5), as well as Dil-Ac-LDL uptake assays are also carried out for each lot. Growth performance is tested through multiple passages up to 15 population doublings (PD) under culture conditions without antibiotics and 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/certificate-of-analysis](http://www.promocell.com/certificate-of-analysis)

### Intended Use

PromoCell Endothelial Cells from large vessels 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 have to be processed immediately (see page 3).*

\*Human Umbilical Artery Endothelial Cells (HUAEC) from the same donor are available on request.

*Use aseptic techniques and a laminar flow bench.*

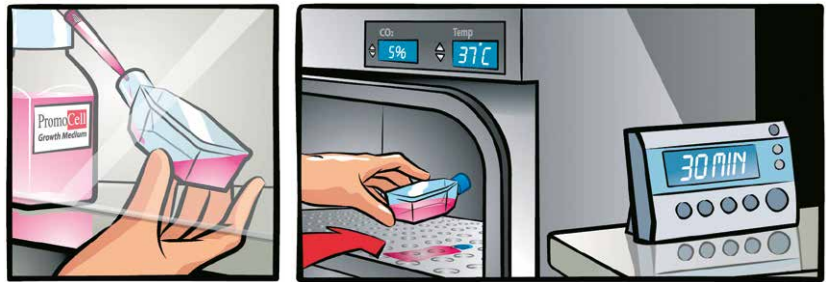
### Protocol for Cryopreserved Cells

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

**Note:** Storage at  $-80^{\circ}\text{C}$  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 ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ) 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 re-tighten. Immerse the vial into a water bath ( $37^{\circ}\text{C}$ ) 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 ( $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ) 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<sub>2</sub>) for 3 hours to allow the cells to recover from the transportation.



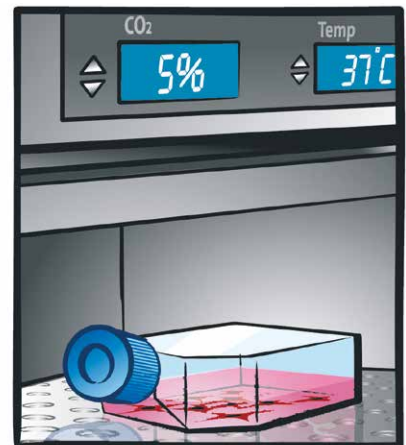
#### 2. Replace the transport medium

Carefully open the vessel, rinse the inner side of the 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<sub>2</sub>). 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.





*Use aseptic techniques and a laminar flow bench.*

## Subcultivation Protocol

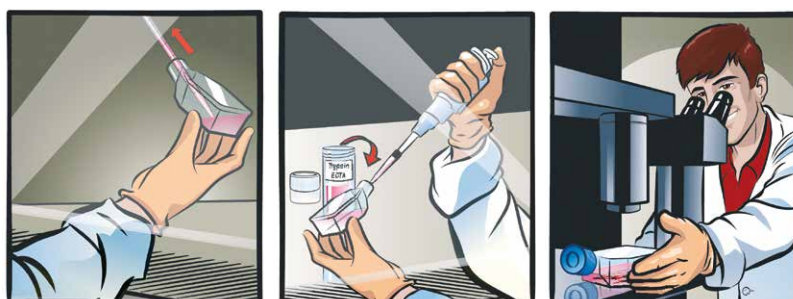
### 1. Prepare the reagents and wash the cells

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  $\mu$ l Hepes BSS Solution per  $\text{cm}^2$  of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



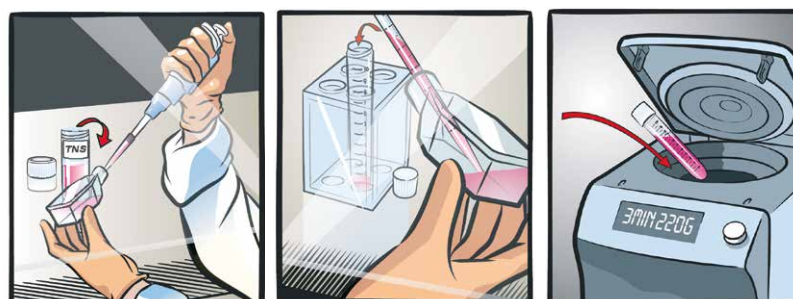
### 2. Detach the cells

Carefully aspirate the Hepes BSS from the culture vessel. Add 100  $\mu$ l Trypsin/EDTA Solution per  $\text{cm}^2$  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.



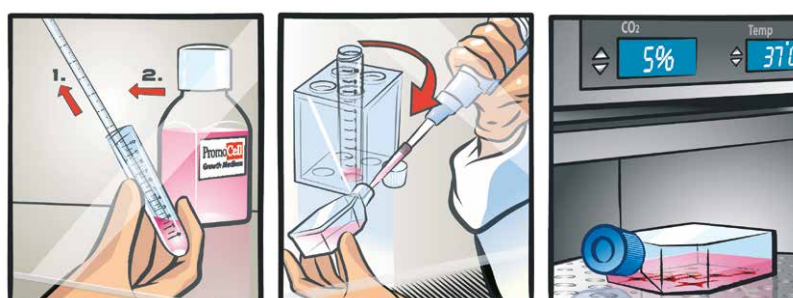
### 3. Neutralize the trypsin and harvest the cells

Add 100  $\mu$ l Trypsin Neutralization Solution per  $\text{cm}^2$  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%  $\text{CO}_2$ ) and change the media every two to three days.



## Specifications

Product	Recommended Culture Media*	Plating Density	Passage after Thawing	Marker	Population Doublings
Human Umbilical Vein Endothelial Cells (HUVEC), single donor	C-22010 C-22011	5,000–10,000 cells per cm <sup>2</sup>	P1	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Umbilical Vein Endothelial Cells (HUVEC), pooled	C-22010 C-22011	5,000–10,000 cells per cm <sup>2</sup>	P1	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, single donor	C-22011	5,000–10,000 cells per cm <sup>2</sup>	P1	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, pooled	C-22011	5,000–10,000 cells per cm <sup>2</sup>	P1	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Umbilical Vein Endothelial Cells (HUVEC), pre-screened	C-22010 C-22011	5,000–10,000 cells per cm <sup>2</sup>	P1	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup> VEGF response	> 15
Human Umbilical Artery Endothelial Cells (HUAEC)	C-22010 C-22011	5,000–10,000 cells per cm <sup>2</sup>	P1	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Aortic Endothelial Cells (HAoEC)	C-22020 C-22022	5,000–10,000 cells per cm <sup>2</sup>	P2	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Coronary Artery Endothelial Cells (HCAEC)	C-22020 C-22022	5,000–10,000 cells per cm <sup>2</sup>	P3	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Pulmonary Artery Endothelial Cells (HPAEC)	C-22010 C-22011	5,000–10,000 cells per cm <sup>2</sup>	P2	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15
Human Saphenous Vein Endothelial Cells (HSAVEC)	C-22010 C-22011	5,000–10,000 cells per cm <sup>2</sup>	P2	vWF <sup>+</sup> CD31 <sup>+</sup> Dil-Ac-LDL uptake <sup>+</sup>	> 15

## Related Products

Product	Size	Catalog Number
Endothelial Cell Growth Medium (Ready-to-use)	500 ml	C-22010
Endothelial Cell Growth Medium Kit	500 ml	C-22110
Endothelial Cell Basal Medium	500 ml	C-22210
Endothelial Cell Basal Medium, phenol red-free	500 ml	C-22215
Endothelial Cell Growth Medium Supplement Mix	for 500 ml	C-39215
Endothelial Cell Growth Medium Supplement Pack	for 500 ml	C-39210
Endothelial Cell Growth Medium 2 (Ready-to-use)	500 ml	C-22011
Endothelial Cell Growth Medium 2 Kit	500 ml	C-22111

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

Product	Size	Catalog Number
Endothelial Cell Basal Medium 2	500 ml	C-22211
Endothelial Cell Basal Medium 2, phenol red-free	500 ml	C-22216
Endothelial Cell Growth Medium 2 SupplementMix	for 500 ml	C-39216
Endothelial Cell Growth Medium 2 SupplementPack	for 500 ml	C-39211
Endothelial Cell Growth Medium MV (Ready-to-use)	500ml	C-22020
Endothelial Cell Growth Medium MV Kit	500ml	C-22120
Endothelial Cell Growth Medium MV 2 (Ready-to-use)	500ml	C-22022
Endothelial Cell Growth Medium MV 2 Kit	500ml	C-22121
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
HUVEC single donor Pellet	1 million cells per pellet	C-14010
HUVEC pooled Pellet	1 million cells per pellet	C-14011
HUVEC Growth Medium 2 single donor Pellet	1 million cells per pellet	C-14008
HUVEC Growth Medium 2 pooled Pellet	1 million cells per pellet	C-14009
HUVEC pre-screened Pellet	1 million cells per pellet	C-14012
HUAEC Pellet	1 million cells per pellet	C-14013
HAoEC Pellet	1 million cells per pellet	C-14023
HCAEC Pellet	1 million cells per pellet	C-14022
HPAEC Pellet	1 million cells per pellet	C-14024
HSaVEC Pellet	1 million cells per pellet	C-14025
PromoFectin-HUVEC	0.1 ml 0.5 ml	PK-CT-2000-HUV-10 PK-CT-2000-HUV-50
VEGF-165, human, recombinant (E. coli)	10 µg	C-64420
VEGF-165, human, recombinant (HEK293)	10 µg	C-64423

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