**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 antifungals. In addition, all cells have been tested for the absence of 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.

A detailed certificate of analysis (CoA) for each lot can be downloaded at: 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!

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

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**Product Size Catalog Number**

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Size</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Umbilical Vein Endothelial Cells (HUVEC) single donor</td>
<td>500,000 cryopreserved cells 500,000 proliferating cells</td>
<td>C-12200 C-12250</td>
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<tr>
<td>Human Umbilical Vein Endothelial Cells (HUVEC) pooled</td>
<td>500,000 cryopreserved cells 500,000 proliferating cells</td>
<td>C-12203 C-12253</td>
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<tr>
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<tr>
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<tr>
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<td>C-12241 C-12242</td>
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<tr>
<td>Human Saphenous Vein Endothelial Cells (HSAVEC)</td>
<td>500,000 cryopreserved cells 500,000 proliferating cells</td>
<td>C-12231 C-12232</td>
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</table>

*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°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% CO₂) 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% CO₂) 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 subcultiva tion 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% CO₂) 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% CO₂). 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% confluence.
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% CO₂) and change the media every two to three days.
### Specifications

<table>
<thead>
<tr>
<th>Product</th>
<th>Recommended Culture Media*</th>
<th>Plating Density</th>
<th>Passage after Thawing</th>
<th>Marker</th>
<th>Population Doublings</th>
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<tbody>
<tr>
<td>Human Umbilical Vein Endothelial Cells (HUVEC), single donor</td>
<td>C-22010 C-22011</td>
<td>5,000 – 10,000 cells per cm²</td>
<td>P1</td>
<td>vWF⁺ CD31⁺ Dil-Ac-LDL uptake⁺</td>
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<tr>
<td>Human Umbilical Vein Endothelial Cells (HUVEC), pooled</td>
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<td>5,000 – 10,000 cells per cm²</td>
<td>P1</td>
<td>vWF⁺ CD31⁺ Dil-Ac-LDL uptake⁺</td>
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<td>5,000 – 10,000 cells per cm²</td>
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<td>5,000 – 10,000 cells per cm²</td>
<td>P1</td>
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<tr>
<td>Human Umbilical Vein Endothelial Cells (HUVEC), pre-screened</td>
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### Related Products

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<tr>
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<tr>
<td>Endothelial Cell Growth Medium Kit</td>
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<tr>
<td>Endothelial Cell Basal Medium</td>
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<tr>
<td>Endothelial Cell Basal Medium, phenol red-free</td>
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<td>C-22215</td>
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<tr>
<td>Endothelial Cell Growth Medium SupplementMix</td>
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<td>C-39215</td>
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<tr>
<td>Endothelial Cell Growth Medium SupplementPack</td>
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<tr>
<td>Endothelial Cell Growth Medium 2 (Ready-to-use)</td>
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<tr>
<td>Endothelial Cell Growth Medium 2 Kit</td>
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*The catalog numbers in this table are for media in ready-to-use packaging.*
<table>
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<th>Product</th>
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<tr>
<td>Endothelial Cell Basal Medium 2, phenol red-free</td>
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<tr>
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