# Human Umbilical Vein Endothelial Cell Care Manual

## INSTRUCTION MANUAL ZBM0079.05

## SHIPPING CONDITIONS

**Human Human Umbilical Vein Endothelial Cells, Cryopreserved**

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

**All orders should be processed immediately upon shipment receipt.**

## STORAGE CONDITIONS

| | | |
|---|---|---|
| **Media:** | +4°C | Expires 30 days from ship date. |
| | -20°C | Expires 6 months from ship date. |
| **Cells:** | Store in vapor phase nitrogen (-150°C to -190°C) IMMEDIATELY UPON RECEIPT. | |

**Any other use negates the warranty.**

***All Zen-Bio Inc. products are for research uses only. Not approved for human or veterinary use or for use in diagnostic or clinical procedures or other uses in humans.***

### ORDERING INFORMATION AND TECHNICAL SERVICES


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| | |
|---|---|
| **Telephone** | +1 (919) 547-0692 |
| **Toll free (continental US only)** | 1-866-ADIPOSE 1-(866)-234-7673 |
| **Electronic mail (e-mail)** | information@zenbio.com |
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### THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

| | |
|---|---|
| HUVEC-F | CRYOPRESERVED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (500,000 cells/vial) |

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## LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Zen-Bio, Inc warrants the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

Contact ZenBio, Inc. within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

## PRECAUTIONS

**This product is for research use only.** It is not intended for human, veterinary, or in vitro diagnostic use. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective screen when handling primary human cells.** All media, supplements, and tissue cultureware used in this protocol should be sterile.

Human umbilical vein endothelial cell (HUVEC) viability depends greatly on the use of suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed, cell growth may be slower than expected.

## INTRODUCTION

Human umbilical vein endothelial cells (HUVECs) are isolated from the vein of the umbilical cord and are commonly used for physiological and pharmacological investigations, such as macromolecule transport, blood coagulation, angiogenesis, and fibrinolysis. HUVECs have played a major role as a model system for the study of the regulation of endothelial cell function and the role of the endothelium in the response of the blood vessel wall to stretch, shear forces, and the development of atherosclerotic plaques.

Each human umbilical cord vein is individually processed (unless otherwise noted for pooled/mixed donor products) to isolate endothelial cells through collagenase digestion and selective cell culture. Frozen HUVEC products are cryopreserved at the end of the primary culture.

Human umbilical vein endothelial cells (HUVECs) from Zen-Bio are obtained from healthy consented adult donors. The HUVECs are obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues.

## QUALITY CONTROL

Each vial of human umbilical vein endothelial cells (HUVECs) contains 500,000 viable cells. The cells are characterized by cell surface marker analysis.

HUVECs are assessed for viability and characterized using flow cytometry for cell surface marker population distributions. HUVECs are expressed as percentage of cells positive for endothelial cell marker CD31 (platelet endothelial cell adhesion molecule-1 (PECAM-1)), von Willebrand factor (vWF), CD146 (cell surface marker protein MUC18), and CD105 (endoglin).

## CATALOG ITEMS

- **Endothelial Cell Growth Medium**
  - Cat # ECGM-1 (500 mL)
  - Store according to label
- **Cryopreserved Human Umbilical Vein Endothelial Cells (HUVECs)**
  - Cat # HUVEC-F
  - Cryopreserved vial containing 500,000 viable HUVECs per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt)** *any other storage negates the warranty*

## MEDIUM COMPOSTION

| **Endothelial Cell Growth Medium** (Cat# ECGM-1) | **Storage and Expiration Date** |
|---|---|
| Minimal Essential Medium - alpha modification + L-Glutamine (α-MEM)<br>Fetal Bovine Serum (FBS; USA Origin)<br>Endothelial Cell Growth Supplement from Bovine Pituitary<br>Porcine Heparin<br>Human Epidermal Growth Factor (hEGF)<br>Basic Fibroblast Growth Factor, Recombinant Human (bFGF)<br>Insulin-like Growth Factor-1 (IGF-1)<br>Vascular Endothelial Cell Growth Factor (VEGF)<br>L-Ascorbic acid 2-phosphate<br>Hydrocortisone<br>Penicilin<br>Stroptomycin<br>Amphotericin B | • If stored at 4°C upon arrival, the media is stable until the expiration date on the bottle.<br>• If stored at -20°C upon arrival, the media is stable for 6 months. *The media will expire 30 days after the thaw date.*<br>• Medium is provided ready to use and prepared fresh prior to shipment. |

# PLATING AND EXPANSION PROCEDURES

## THAWING AND CULTURING HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

*Note: Primary human cell viability is greatly dependent on the use of appropriate sterile tissue culture treated cultureware. Zen-Bio recommends the use of gelatin coated cultureware or ZenBio brand Collagen I coated cultureware. See FAQ for details.*

*Note: HANDLE GENTLY AND QUICKLY TO MAINTAIN VIABILITY.*

1. Pre-warm Endothelial Cell Growth Medium (cat# ECGM-1) at 37°C, and prepare all pipets and gelatin or collagen coated vessels.
2. Transfer 9.5 mL of warm ECGM-1 to a sterile 15 mL conical centrifuge tube.
3. Remove cryovial of human umbilical vein endothelial cells (HUVECs) from liquid nitrogen and place **immediately** into a 37°C water bath with mild agitation. Be careful not to submerge the cap of the vial into water. For best results, the thawing step should not take more than 1 minute, and should be stopped when there is still visible ice within the vial.
4. Rinse cryovial with 70% ethanol, and wipe cryovial with lint-free lab wiper. Open cryovial under laminar flow hood and resuspend cells in previously prepared 9.5 mL of warmed ECGM-1.
5. Centrifuge cell suspension at 300 x *g* for 5 minutes at 20ºC.
6. Carefully aspirate the supernatant, being careful not to disturb the cell pellet, and resuspend in a volume of ECGM-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
7. The plating density of HUVECs is 5,000 cells per $cm^2$ for expansion. Calculate the necessary culture surface area according to the plating density (being sure to reference the manufacturer specifications for cell culture area).
8. Place vessel in an incubator (37°C, 5% $CO_2$) for cell attachment. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate the plates or flasks after plating, making sure the vessel surface is level for even cell distribution. For the best results, do not disturb the culture vessel for at least 12 hours after plating.
9. Replace medium after 16-24 hours. Medium should be changed every other day until cells reached 85-90% confluency.

### *OPTIONAL* – HUMAN UMBILICAL VEIN ENDOTHELIAL CELL (HUVEC) SUBCULTURE

*Note: HUVECs should not be expanded after passage 7.*

1. HUVECs should be 85-90% confluent upon harvest for expansion.
2. Pre-warm Endothelial Cell Growth Medium (cat# ECGM-1) to 37ºC, letting the Dulbecco's phosphate buffered saline without calcium or magnesium (cat# DPBS-100) and the Trypsin/EDTA solution (cat# TRP-100) warm to room temperature, and prepare all pipets and gelatin or collagen coated vessels.
3. Carefully aspirate medium from cell culture vessel and wash cells using sterile DPBS-1000 to remove all traces of serum, or until there is no foaming of the medium.
4. Remove the DPBS-1000 and release the cells from the cultureware bottom by adding Trypsin/EDTA solution at 0.1-0.2 mL per $cm^2$ cultureware surface area.
5. Incubate cells for 5-10 minutes at 37°C if using Trypsin/EDTA.
6. Examine cells under microscope, and once cells begin detaching, gently tap the side of the vessel to loosen the remaining cells.

7. Neutralize Trypsin/EDTA solution using 0.1-0.2 mL ECGM-1 per $cm^2$ cultureware surface area. Carefully transfer the cell suspension to a conical bottom centrifuge tube.
8. Centrifuge cell suspension at 300 x *g* for 5 minutes at 20ºC.
9. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and resuspend the in a volume of ECGM-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
10. Seed cells at 5,000 cells per $cm^2$ in the appropriate gelatin coated or collagen I coated vessel, and place vessel in an incubator (37°C, 5% $CO_2$) for cell attachment. Ensure cells are evenly suspended when plating large numbers of plates or flasks. Do not agitate the plates or flasks after plating, making sure the vessel surface is level for even cell distribution. For the best results, do not disturb the culture vessel for at least 12 hours after plating.
11. Replace the medium every 2-3 days. Once cells have reached 85-90% confluency they should be subcultured further or harvested and cryopreserved.

# CRYOPRESERVATION PROCEDURE

1. Human umbilical vein endothelial cells (HUVECs) should be 85- 90% confluent upon harvest for cryopreservation.
2. Aspirate medium and wash cells using sterile Dulbecco's phosphate buffered saline without calcium or magnesium (cat# DPBS-1000) to remove all traces of serum, or until there is no foaming of the medium.
3. Remove the DPBS-1000 and release the cells from the cultureware bottom by adding Trypsin/EDTA solution (cat# TRP-100) or Accutase® Cell Detachment Solution at 0.1-0.2 mL per $cm^2$ cultureware surface area.
4. Incubate cells for 5-10 minutes at 37°C if using Trypsin/EDTA, or incubate cells for 5-10 minutes at room temperature if using Accutase® Cell Detachment Solution.
5. Examine cells under microscope, and once cells begin detaching, gently tap the side of the vessel to loosen the remaining cells.
6. Neutralize Trypsin/EDTA solution by adding Endothelial Cell Growth Medium (cat# ECGM-1) at 0.1-0.2 mL per $cm^2$ cultureware surface area; no inhibitors or washing solution is needed for Accutase® Cell Detachment Solution. Carefully transfer the cell suspension to an appropriate centrifuge tube.
7. Centrifuge cell suspension at 300 x *g* for 5 minutes at 20ºC.
8. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and resuspend the in a volume of ECGM-1 appropriate for counting the cells. Count cells using a hemocytometer or automated cell counter.
9. Centrifuge cell suspension at 300 x *g* for 5 minutes at 20ºC.
10. Carefully aspirate supernatant, being careful not to disturb the cell pellet, and suspend in cold Cryostor CS10 at a concentration of 1 million cells per mL. Do not exceed a 6:1 ratio of cells (per million) to volume cryopreservation medium (per mL). Remember to account for the volume of the cell pellet before adding the volume of cryopreservation medium necessary for cell suspension.
11. If using a controlled-rate freezer: Freeze by reducing the temperature 1°C per minute until the temperature reaches -80° C. If using a cell cryopreservation container, prepare according to the manufacturer's instructions.
12. For best results we recommend transferring the vials to the vapor phase of a liquid nitrogen storage facility as soon as possible after the cells have reached -80°C.

# FREQUENTLY ASKED QUESTIONS

**How far can I expand the cells?**

We do not recommend using the human umbilical vein endothelial cells (HUVECs) after passage 7. HUVECs are sold at passages 2-5 after establishing a primary culture.

**Why are my cells not attaching?**

Primary HUVECs require the use of gelatin coated or collagen I coated cultureware.

**Is there a specific type of cultureware that should be used?**

Yes. Primary HUVECs require the use of gelatin or collagen coated cultureware. If you are using collagen coated cultureware, only Zen-Bio Collagen I Coated Cultureware should be used (catalogue numbers below).

| Item# | Item Description |
|---|---|
| CC-6 | Collagen I Coated 6-well Plate, Pack of 5 |
| CC-12 | Collagen I Coated 12-well Plate, Pack of 5 |
| CC-24 | Collagen I Coated 24-well Plate, Pack of 5 |
| CC-48 | Collagen I Coated 48-well Plate, Pack of 5 |
| CC-96 | Collagen I Coated 96-well Plate, Pack of 5 |
| CC-384 | Collagen I Coated 384-well Plate, Pack of 5 |
| CC-25 | Collagen I Coated T-25 Flask, Vent Cap, Pack of 5 |
| CC-75 | Collagen I Coated T-75 Flask, Vent Cap, Pack of 5 |
| CC-175 | Collagen I Coated T-175 Flask, Vent Cap, Pack of 5 |
| CC-225 | Collagen I Coated T-225 Flask, Vent Cap, Pack of 1 (EXCLUSIVE!) |

**Should antibiotics be included in the medium?**

Yes. Antibiotics and anti-fungal agents are always recommended for primary cells such as HUVECs.

**Are the cells tested for any blood borne pathogen?**

Yes. See the section titled Pathogen Testing below for more details.

# PATHOGEN TESTING

Samples from each donor are tested via PCR and found non-reactive to viral DNA from HIV and Hepatitis B and viral RNA from Hepatitis C. However, no known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially infectious reagent. We recommend using the US Centers for Disease Control (CDC) Universal Precautions for prevention of blood-borne pathogens as a minimum guideline for standards of practice. Our products are tested for mycoplasma contamination. Mycoplasma is not detected in our labs. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-1 (Biosafety Level 1) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.