Human Umbilical Vein Endothelial Cell Manual

INSTRUCTION MANUAL ZBM0079.03

SHIPPING CONDITIONS

Human Umbilical Vein Endothelial Cells, cryopreserved

Cryopreserved cells are shipped on dry ice and should be stored in vapor phase liquid nitrogen immediately upon arrival. Orders are delivered via overnight or priority courier.

Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Medium: Store at 2-8°C only *NOTE: Expiration date is 30 days from the ship date.*

Cryopreserved cells: Cells are to be stored in vapor phase liquid nitrogen (-150°C to -190°C) IMMEDIATELY upon receipt.

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

ORDERING INFORMATION AND TECHNICAL SERVICES

ZenBio, Inc.
3200 East NC Highway 54, Suite 100
PO Box 13888
Research Triangle Park, NC 27709
U.S.A.

Telephone	(919) 547-0692
Facsimile (FAX)	(919) 547-0693
Toll free (continental US only)	1-866-ADIPOSE 1-(866)-234-7673
Electronic mail (e-mail)	information@zenbio.com
World Wide Web	http://www.zenbio.com

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 its cells only if Zen-Bio media are used and the recommended protocols are followed. Cryopreserved human umbilical vein endothelial cells (HUVEC) are assured to be viable when thawed according to Zen-Bio protocols.

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 culture ware used in this protocol should be sterile.

To comply with U.S. Food and Drug Administration (FDA) regulations, these products are not for use in Clinical Diagnostic or Therapeutic Procedures.

By your acceptance of these products, you are acknowledging that these products will be:

1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative;
2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

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.

QUALITY CONTROL

Human Umbilical Vein Endothelial Cells from Zen-Bio are obtained from healthy consented adult donors. Each vial of HUVECs contains 500,000 viable cells. The cells are characterized by cell surface marker analysis.

Each lot of primary cells is 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.

Human Umbilical Cell Endothelial Cells (HUVEC) are 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).

MATERIALS PROVIDED FOR EACH CATALOG ITEM_____

***Note**: Zen-Bio recommends that all cells be processed immediately upon receipt.*

Cryopreserved human umbilical vein endothelial cells, ≥500,000 cells/vial, cat# HUVEC-F
-50ml Endothelial Cell Growth Medium (cat# ECGM-1)
NOTE: This media expires 30 days from the ship date. DO NOT FREEZE

MEDIUM COMPOSTION _____

Endothelial Cell Growth Medium (ECGM-1)

Minimal Essential Medium- alpha modification + L-Glutamine (α-MEM)
Fetal Bovine Serum
Endothelial Cell Growth Factor Supplement from bovine pituitary
Porcine Heparin
Endothelial Cell Growth Factor, human (hEGF) Basic Fibroblast Growth Factor (bFGF)
Insulin-like Growth Factor-1 (IGF-1)
Vascular Endothelial Cell Growth Factor (VEGF) Ascorbic Acid
Hydrocortisone
Penicillin
Streptomycin
Amphotericin B

NOTE:
All media are provided ready to use and prepared fresh prior to shipment.
The expiration date is 30 days from the ship date.
Please schedule your orders accordingly.

THAWING AND PLATING CRYOPRESERVED HUVECs

NOTE: HANDLE GENTLY AND QUICKLY TO MAINTAIN VIABILITY.

WE RECOMMEND THE USE OF GELATIN COATED CULTUREWARE AT ALL TIMES

Instructions for seeding Human umbilical vein endothelial cells

1. Place vial in a 37˚C water bath, hold and rotate vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe dry, rinse the vial with 70% ethanol and transfer to sterile field. Remove cap, being careful not to touch the interior threads with fingers.
2. Using a pipette, gently transfer contents of vial to a 15 ml conical tube. Wash vial with 5 ml medium and add wash to conical tube.
3. Centrifuge tube at 250g for 5 minutes. After centrifugation, aspirate medium and re-suspend the contents in medium. Perform a cell count.
4. For expansion, seed the cells at a density of 5000 cells/cm^2 on gelatin coated plates.
5. For best results, do not disturb the culture for at least 12 hours after seeding.
6. Change growth medium the next day to remove any residual DMSO or unattached cells, then every feed fresh growth media every other day thereafter.

Instructions for sub-culturing HUVECs

1. Subculture cells when they have reached 85-90% confluency.
2. Warm medium, 0.25% trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS) to room temperature.
3. Aspirate medium, then rinse cells with DPBS. Add trypsin solution into flask and incubate in a 37˚C incubator for 3-5 minutes, or until the cells detach.
4. At the end of typsinization, wash cells off flask with an appropriate amount of medium. Transfer to centrifuge tube, centrifuge at 250g for 5 minutes. After centrifugation aspirate medium, re-suspend and count cells for seeding.
5. Seed the cells at a density of 5,000 cells/cm^2 using gelatin coated cultureware

FREQUENTLY ASKED QUESTIONS

1. **How far can I expand the cells?**
   We do not recommend using the HUVEC beyond passage 7. HUVEC are sold at passages 2-5 after establishing a primary culture.

2. **Why are my cells not attaching?**
   Primary HUVEC require the use of gelatin coated cultureware.

3. **Are the cells tested for any blood borne pathogen?**
   Yes. Samples from each donor are tested via PCR to confirm non-reactivity for HIV, hepatitis B, and hepatitis C. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent.

4. **Can I freeze the medium to extend the shelf life?**
   No. Media should be stored at all times at 2-8°C only. The media expires 30 days from the shipping date. Please coordinate the timing of your media shipment with your experiments. Notify ZenBio at the time of order if you have a preferred schedule for the delivery that best meets your experimental needs.

5. **Should antibiotics be included in the medium?**
   Yes. Antibiotics and anti-fungal agents are always recommended since the cells are primary cells.

株式会社ケー・エー・シー
試薬事業部
〒661-0978　兵庫県尼崎市久々知西町2丁目1-20

（お問合せ先）
TEL：06-6435-9747　FAX：06-6435-9748
URL：https://www.kacnet.co.jp/
E-mail：shiyaku-info@kacnet.co.jp

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