



# Human Hepatic Sinusoidal Endothelial Cell Manual

## INSTRUCTION MANUAL ZBM0093.00

### SHIPPING CONDITIONS

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**Human Hepatic Sinusoidal Cells.** Orders are delivered via Federal Express courier. All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are usually received in 2-4 days.

**Must be processed upon shipment receipt.**

### STORAGE CONDITIONS

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**Media:** Store as indicated IMMEDIATELY UPON ARRIVAL

- Endothelial Cell Growth Medium: Store at +4°C; DO NOT FREEZE

**Cells:** Human Hepatic Sinusoidal cells are to be stored in vapor phase 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.***

### 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 without amendment or substitution.

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.

### Ordering Information and Technical Services

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## PRECAUTIONS

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**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 hepatic sinusoidal endothelial cell viability depends greatly on the use of the recommended protocols, suitable media, reagents, and sterile plastic wear. If these parameters are not carefully observed this may result in poor growth, viability and differentiation capacity of the cells.

## INTRODUCTION

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Human hepatic sinusoidal endothelial cells (HHSECs) are isolated from human liver obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation. They are isolated through collagenase digestion and selective cell culture. These cells are a valuable tool for the study of liver physiology and pathophysiology. They are specialized endothelial cells that participate in receptor-mediated clearance of endotoxin, bacteria, and other compounds. They also regulate inflammation, leukocyte recruitment, and host immune responses to pathogens. Frozen HHSECs are cryopreserved at the end of the primary culture

## QUALITY CONTROL

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Human Hepatic Sinusoidal Endothelial Cells from Zen-Bio are obtained from donor tissue. Each vial of HUVECs contains 500,000 cells. The cells are characterized by a panel of markers to verify cell type.

Each lot is tested via PCR and found non-reactive to viral DNA from HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B and hepatitis C. HHSECs are characterized using flow cytometry for population distributions. HHSECs are positive for CD105, von Willebrand Factor, DIL-AC-LDL, CD31, and CD146.

## **MATERIALS PROVIDED FOR EACH CATALOG ITEM**

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- **Cryopreserved Human Hepatic Sinusoidal Endothelial Cells**

- Cat # HSEC-F
- Frozen vial containing  $\geq 500,000$  viable cells
- 50ml Endothelial Cell Growth Medium (ECGM-1) per vial (*NOTE: may be combined in larger format when multiple vials are ordered*)

Store in vapor phase nitrogen (-150°C to -190°C) immediately upon receipt

## **MEDIUM COMPOSITION**

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### **Endothelial Cell Growth Medium Cat# ECGM-1**

Note: This medium has been developed to optimize to maintain hepatic sinusoidal cells.

Storage: +4°C

Composition:

- Minimal Essential Medium- alpha modification + L-Glutamine ( $\alpha$ -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

## **THAWING AND PLATING CRYOPRESERVED HHSECS**

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### **Instructions for seeding Human hepatic sinusoidal 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<sup>2</sup> on Collagen I coated plates.
5. For best results, do not disturb the culture for at least 12 hours after seeding. Change growth medium the next day to remove any residual DMSO or unattached cells, then every other day thereafter.

### **Instructions for sub-culturing HHSECs**

1. Subculture cells when they have reached 70-80% 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 trypsinization, 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<sup>2</sup> on collagen I coated plates.

## **FREQUENTLY ASKED QUESTIONS**

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1. Can I passage the cells?
  - a. Cells are shipped at passage 3 after establishing a primary culture. We guarantee performance up to 2 passages (passage 5). Do not exceed passage 5.
2. How fast do the cells replicate?
  - a. The average doubling time is 48-60 hours. However, keep in mind that the replication rate for human hepatic sinusoidal cells varies from donor to donor.
3. Is there a specific type of cultureware that should be used?
  - a. Yes.
  - b. Only BD Biocoat brand or ZenBio Collagen Coated Cultureware should be used.

### **Zen-Bio Collagen Coated Cultureware**

<b>Item#</b>	<b>Item Description</b>
CC-25	Collagen Coated I T-25 Flask, Vent Cap, Pack of 5
CC-75	Collagen Coated I T-75 Flask, Vent Cap, Pack of 5
CC-225	Collagen Coated I T-225 Flask (EXCLUSIVE!), Vent Cap, Pack of 1
CC-6	Collagen Coated I 6-well Plate, Pack of 5
CC-12	Collagen Coated I 12-well Plate, Pack of 5
CC-24	Collagen Coated I 24-well Plate, Pack of 5
CC-96	Collagen Coated I 96-well Plate, Pack of 5

4. Where are the cells obtained?
  - a. The human hepatic sinusoidal cells are from human liver obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation.
5. Do you test for pathogens? Which ones?
  - a. Yes.
  - b. Samples from each donor are tested for HIV 1 and II, human T lymphotropic virus I and II, Hepatitis B surface antigen and core antibody, Hepatitis C antibody, syphilis, and cytomegalovirus. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent.

## **PATHOGEN TESTING**

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Samples from each donor are tested via PCR to confirm non-reactivity for HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B, hepatitis C, syphilis, and CMV. However, no known test can offer complete assurance that the cells are pathogen free. Our products are tested and are free from mycoplasma contamination. 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-2 (Biosafety Level 2) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.