Human Hepatic Sinusoidal Endothelial Cell Manual

INSTRUCTION MANUAL ZBM0093.03

SHIPPING CONDITIONS

Human Hepatic Sinusoidal Cells.

All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are shipped using dry ice or using a dry vapor shipper if transit time will exceed 3 days. Hepatic cells are very sensitive to extended times (> 3 days) transported using dry ice. Please inquire for dry vapor shipper availability if your transit time will exceed 3 days. Cells should always be stored in liquid nitrogen vapor phase immediately upon arrival. **Must be processed immediately upon shipment receipt.**

STORAGE CONDITIONS

Media: 30 days from ship date 4^0C 6 months -20^0C

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.

Ordering Information and Technical Services

ZenBio, Inc.
3920 South Alston Avenue
Durham, NC 27713
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

THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

HSEC-F	HUMAN HEPATIC SINUSOIDAL ENDOTHELIAL CELLS, CRYOPRESERVED, 500,000 CELLS/VIAL

LIMITED PRODUCT WARRANTY

This warranty limits our liability for 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 and storage conditions are followed. Cryopreserved cells are assured to be viable when thawed according to Zen-Bio protocols and using the recommended cultureware.

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 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 results in poor growth, viability and differentiation capacity of the cells.

CONTENTS

INTRODUCTION

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. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues. The cells are isolated through collagenase digestion and selective cell culture media. 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

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

All donor lots are screened for HIV-1, HIV-2, Hepatitis B, Hepatitis C, syphilis, Cytomegalovirus (CMV), and Epstein Barr Virus (EBV) using US Food and Drug Administration (FDA) licensed tests. However, no known test can offer complete assurance these viruses are not present. Since we cannot test all the pathogens, please handle the culture as a potential infectious reagent at Biosafety Level 1 or higher. 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.

HSECs are assessed for viability and characterized using flow cytometry for population distributions of cell surface markers. HHSECs are positive for CD105, von Willebrand Factor, CD31, and CD146.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Hepatic Sinusoidal Endothelial Cells**
  - Cat # HSEC-F
  - Frozen vial containing $\geq$ 500,000 viable cells

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

MEDIUM COMPOSITION

Endothelial Cell Growth Medium Cat# ECGM-1
Note: This medium has been developed to optimize to maintain hepatic sinusoidal cells.

Endothelial Cell Growth Medium Cat# ECGM-1	**EXPIRATION DATE**
• Minimal Essential Medium- alpha modification + L-Glutamine (α-MEM) • Fetal Bovine Serum (FBS) • 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	• If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label. • If stored at -20°C upon arrival, it is stable for 6 months. • Add fresh antibiotics at 1% concentration by volume, after thawing and you are ready to use.
Endothelial Cell Cryopreservation Medium Cat# ECCM-1	
Fetal Bovine Serum, US origin (FBS) Dimethyl sulfoxide (DMSO)	Use expiration date listed on bottle.

THAWING AND PLATING CRYOPRESERVED HSECs

NOTE: THAWED CELLS ARE FRAGILE. HANDLE GENTLY AND QUICKLY TO MAINTAIN VIABILITY.
ZEN-BIO RECOMMENDS THE USE OF CORNING BIOCOAT® OR ZENBIO BRAND COLLAGEN I CULTUREWARE SEE FAQ FOR DETAILS

A. 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 250 x g for 5 minutes. After centrifugation, aspirate medium and re-suspend the contents in medium.
4. Count the cells.
5. For expansion, seed the cells at a density of 5,000 cells/cm^2 on Collagen I coated plates.
6. 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.
7. Continue to change media every other day until ready for use.

B. Instructions for sub-culturing HHSECs

1. Subculture cells when they have reached 70-80% confluency.
2. Warm ECGM-1 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 5.0 ml trypsin solution into a 75cm^2 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 ECGM-1 medium.
5. Transfer the cells to centrifuge tube, centrifuge at 250 x g, room temperature for 5 minutes.
6. After centrifugation aspirate medium, re-suspend and count cells for seeding.
7. Seed the cells at a density of 5,000 cells/cm^2 on collagen I coated plates.

FREQUENTLY ASKED QUESTIONS

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. Can I re-freeze the cells?
    a. If you thaw and use a small portion of the cells, you must plate the cells for at least 24 hours before re-freezing.
    b. We recommend our Endothelial Cell Cryopreservation Medium, cat# ECCM-100, for use with the cells.

3. 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.

4. Is there a specific type of cultureware that should be used?
    a. Yes.
    b. Only Corning Biocoat brand or ZenBio Collagen Coated Cultureware should be used.

Zen-Bio Collagen I Coated Cultureware

Item#	Item Description
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

5. From 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. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues.

6. Do you test for pathogens? Which ones?
    a. Yes. Samples from each donor are tested for HIV-1, HIV-2, Hepatitis B, Hepatitis C, syphilis, Cytomegalovirus (CMV), and Epstein Barr Virus (EBV) using US Food and Drug Administration (FDA) licensed tests. However, no known test can offer complete assurance these viruses are not present. Since we cannot test all the pathogens, please handle the culture as a potential infectious reagent at Biosafety Level 1 or higher.

PATHOGEN TESTING

Samples from each donor are tested for HIV-1, HIV-2, Hepatitis B, Hepatitis C, syphilis, Cytomegalovirus (CMV), and Epstein Barr Virus (EBV) using US Food and Drug Administration (FDA) licensed tests. However, no known test can offer complete assurance these viruses are not present. Since we cannot test all the pathogens, please handle the culture as a potential infectious reagent at Biosafety Level 1 or higher. 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. 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.