



# Human Intrahepatic Biliary Epithelial Cell Manual

## INSTRUCTION MANUAL ZBM0094.02

### SHIPPING CONDITIONS

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**Human Intrahepatic Biliary Epithelial 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

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

**Cells:** Human Intrahepatic Biliary Epithelial 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 culture ware used in this protocol should be sterile.

Human intrahepatic biliary epithelial 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 Intrahepatic Biliary epithelial cells (IHBEc) are isolated from human liver obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation. IHBEcs are the epithelial cells that line the intrahepatic bile ducts. These cells are important in modification of the ductal bile and are targeted in multiple liver diseases, such as primary biliary cirrhosis, cholangiocarcinoma and sclerosing cholangitis. Human Intrahepatic Biliary Epithelial Cells are cryopreserved at the end of the primary culture.

## QUALITY CONTROL

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Human Intrahepatic Biliary Epithelial Cells from Zen-Bio are obtained via the gift of organ donation. Each vial of IHBEcs 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, hepatitis B and hepatitis C. IHBEcs are characterized using flow cytometry for population distributions. IHBEcs are positive for Cytokeratin 18, Cytokeratin 19, and Vimentin.

## MATERIALS PROVIDED FOR EACH CATALOG ITEM

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- **Cryopreserved Human Intrahepatic Biliary Epithelial Cells**
    - Cat # IHBEc-F
    - Frozen vial containing 500,000 viable human intrahepatic biliary epithelial cells.
    - 50 ml Intrahepatic Biliary Cell Medium (IHBEc-1)
- Store cells in vapor phase nitrogen (-150°C to -190°C) immediately upon receipt

## **MEDIUM COMPOSITION**

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### **Intra-Hepatic Biliary Epithelial Cell Growth Medium Cat# IHBEC-1**

Note: This medium has been developed to optimize to maintain intrahepatic biliary epithelial cells.

Storage: +4°C. DO NOT FREEZE.

Composition:

- Minimal Essential Medium, alpha modification
- Fetal Bovine Serum (FBS)
- Epidermal Growth Factor, human (hEGF)
- Hydrocortisone
- Vascular Endothelial Growth Factor (VEGF)
- Fibroblast Growth Factor-Basic, Human (bFGF)
- Ascorbic Acid
- Heparin
- R3-Insulin-like Growth Factor, Long Factor R3 (IGF-1)
- Penicillin
- Streptomycin
- Amphotericin B

#### **NOTE:**

Medium is provided ready to use and prepared fresh prior to shipment.

The expiration date is 30 days from the ship date. DO NOT FREEZE.

Please schedule your orders accordingly.

## **THAWING AND PLATING CRYOPRESERVED IHBECS**

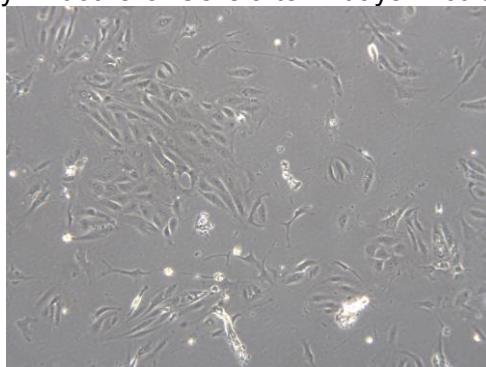
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### **Instructions for seeding Human intrahepatic biliary cells:**

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 a 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 IHBEC-1 medium and add the wash to the same conical tube.
3. Centrifuge tube at 250xg 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 5,000 cells/cm<sup>2</sup> on ZenBio, Inc. or Corning/BD brand 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.
6. Feed cells fresh IHBEC-1 medium every other day until ready for assay or expansion.

**Instructions for sub-culturing IHBECS**

1. Subculture cells when they have reached 70 - 80% confluency.
2. Warm IHBEC-1 medium in a 37°C water bath.
3. Make sure 0.25% trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS) are at room temperature.
4. Aspirate the 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.
5. As soon as the cells detach, wash cells from flask using two (2) times the volume with IHBEC-1 medium. Transfer to centrifuge tube, centrifuge at 250xg for 5 minutes. After centrifugation aspirate medium, re-suspend and count cells for seeding.
6. Seed the cells at a density of 5,000 cells/cm<sup>2</sup> in collagen I coated plates.
7. Figure 1. Intrahepatic Biliary Endothelial Cells after 4 days in culture

**FREQUENTLY ASKED QUESTIONS**

1. Is there a specific type of culture ware that should be used?
  - a. Yes.
  - b. Only Corning/BD Biocoat or ZenBio brand Collagen I Coated Cultureware should be used.

**ZenBio Collagen Coated Culture ware**

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

2. How many times can I passage the cells?
  - a. You may passage the cells 2 times.
3. Do you test for pathogens? Which ones?
  - a. Yes.
  - b. Samples from each donor are tested for Human Immunodeficiency Virus (HIV) Hepatitis B surface antigen and core antibody, and Hepatitis C antibody. However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent using Biosafety Lever 2 or higher.

## 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, and hepatitis C. 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.

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