



Human Intrahepatic Biliary Epithelial Cell Manual

INSTRUCTION MANUAL ZBM0094.04 SHIPPING CONDITIONS

Human Intrahepatic Biliary Epithelial 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 the total transit time will exceed 3 days. Primary Human cells can be 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. Orders must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media: 1 month from ship date 4°C 6 months from ship date -20°C

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.

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

IHBEC-F	HUMAN INTRA-HEPATIC BILIARY EPITHELIAL CELLS: 500,000 CELLS/VIAL
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Zen-Bio, Inc warrants its cells only if Zen-Bio media are used and the recommended protocols and storage conditions are followed without amendment or substitution.

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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.

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

Human Intrahepatic Biliary Epithelial Cells (IHBE) 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.

ZenBio, Inc. Primary Human Intrahepatic Biliary Epithelial Cells (IHBE) 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 IHBE are cryopreserved at the end of the primary culture.

QUALITY CONTROL

All donor lots are found to be negative for HIV-1, HIV-2, Hepatitis B, and Hepatitis C via US Food and Drug Administration (FDA) licensed tests. However, no known test can offer complete assurance that the viruses that these infectious diseases are not present. Since we cannot test all the pathogens, please treat 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. Our products are tested and are free from mycoplasma contamination.

Cells are assessed for viability and cell surface marker analysis via flow cytometry. The traditional epithelial cell surface markers EpCam (CD326), Cytokeratin 8, 18, 19 (CK 8, 18, 19) pan cytokeratins AE1/AE3 (Pan CK AE1/AE3), and the liver-specific marker gamma-glutamyltransferase (GGT-1) are assessed and the data expressed as percentage positive for each marker. Read the certificate of analysis for each lot for details.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Intrahepatic Biliary Epithelial Cells**

- Cat # IHBEC-F
- Frozen vial containing 500,000 viable human intrahepatic biliary epithelial cells.

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

MEDIUM COMPOSITION

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: 1 month 4°C 6 months -20°C

Medium Composition:

- Dulbecco's Modified Eagle's Medium (DMEM)
- MCDB153 Medium
- Fetal Bovine Serum (FBS)
- Epidermal Growth Factor, Recombinant Human (rhEGF)
- Bovine Pituitary Extract (BPE)
- Penicillin
- Streptomycin
- Amphotericin B

NOTES:

- Medium is provided ready to use and prepared fresh prior to shipment.
- 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, the media is stable for 6 months. Add fresh antibiotics when you are ready to use. The media will expire 30 days after the thaw date.

THAWING AND PLATING CRYOPRESERVED IHBECS

NOTE: HANDLE GENTLY AND QUICKLY TO MAINTAIN VIABILITY.

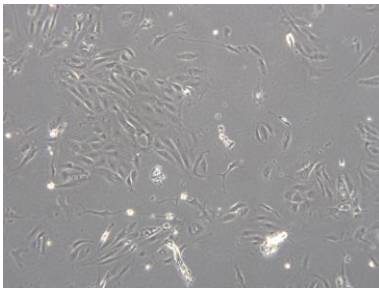
ZEN-BIO RECOMMENDS THE USE OF CORNING BIOCOAT® OR ZENBIO BRAND CULTUREWARE SEE FAQ FOR DETAILS

A. 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/cm² 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.

B. 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/cm² in collagen I coated plates.
7. Figure 1. Intrahepatic Biliary Endothelial Cells after 4 days in culture



C. CRYOPRESERVATION PROCEDURE

1. Aspirate medium and wash cells 4-5 times using sterile Dulbecco's Phosphate Buffered Saline without calcium or magnesium (cat# DPBS-1000) to remove all traces of serum (until there is no foaming of the medium).
2. Remove the PBS and release the cells from the culture ware bottom by adding 2 ml/T-75 flask of 0.25% trypsin/ 2.21 mM EDTA solution cat# TRP-100).
3. Incubate cells with trypsin solution for 5 minutes at 37°C.
4. Neutralize the trypsin using 0.1 ml IHBE-1 Medium per cm² culture ware surface area (e.g. 7.5 ml for T-75 flask). Check under a microscope to ensure all cells are liberated.
5. Centrifuge at 280 x g, 20°C, 5 minutes. Aspirate the medium and suspend cells in a volume of PM-1 appropriate for counting the cells. Count using a hemocytometer.
6. Centrifuge at 280 x g, 20°C, 5 minutes. Suspend in cold cryopreservation medium at a concentration of 1X10⁶ cells/ml.
7. Do not exceed a 6:1 ratio of cells (per million): 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.
8. 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.
9. 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 (FAQ)

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

Catalog#	Item Description	Unit
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 Level 1 or higher.
4. Can I freeze the cells for later use?
 - a. You may cryopreserve the cells for later use. Read Section C of the Cryopreservation Procedure on page 5 for instructions.

PATHOGEN TESTING

Samples from each donor lot are found to be negative for HIV-1, HIV-2, Hepatitis B, and Hepatitis C via US Food and Drug Administration (FDA) licensed tests. However, no known test can offer complete assurance that the viruses that these infectious diseases are not present. Since we cannot test all the pathogens, please treat 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. Our products are tested and are free from mycoplasma contamination. Always wear appropriate personal protective equipment (PPE) including gloves and work behind a protective screen when handling primary human cells.