



Human Intrahepatic Biliary Epithelial Cell Manual

INSTRUCTION MANUAL ZBM0094.00

SHIPPING CONDITIONS

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

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.

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

INTRODUCTION

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

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-1, HIV-2, HTLV I, HTLV II, 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

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

MEDIUM COMPOSITION

Epithelial Cell Growth Medium Cat# IHBE-1

Note: This medium has been developed to optimize to maintain intrahepatic biliary epithelial cells.
Storage: +4°C. DO NOT FREEZE.

Composition:

- DMEM- high glucose (4.5g/L)
- Fetal Bovine Serum (FBS)
- Penicillin
- Streptomycin
- Amphotericin B

THAWING AND PLATING CRYOPRESERVED IHBEs

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 IHBE-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 IHBE-1 medium every other day until ready for assay or expansion.

Instructions for sub-culturing IHBEs

1. Subculture cells when they have reached 70 - 80% confluency.
2. Warm IHBE-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 IHBE-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

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 Culture ware should be used.

Zen-bio 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. Do you test for pathogens? Which ones?
 - a. Yes.
 - b. Samples from each donor are tested for Human Immunodeficiency Virus (HIV) 1 and II, human T lymphotropic virus (HTLV) I and II, 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 2 or higher.

PATHOGEN TESTING ---

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.