Human Hepatocytes Care Manual

INSTRUCTION MANUAL ZBM0003.08

SHIPPING CONDITIONS

Human Hepatocytes cryopreserved

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 total transit time will exceed 2-3 days). Hepatic cells are very sensitive to extended times (> 2-3 days) transported using dry ice. Please inquire for dry vapor shipper availability. Cells should always be stored in liquid nitrogen vapor phase immediately upon arrival. **Must be processed immediately upon shipment receipt.**

STORAGE CONDITIONS

Media: HM-1 Medium: +4° expires 30 days from ship date
-20°C expires 6 months from ship date
HM-2 Medium: +4°C Expires 30 days from ship date. DO NOT FREEZE.

Cells: Store 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

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

HP-F	HUMAN HEPATOCYTES, CRYOPRESERVED, PLATEABLE
HP-NP	HUMAN HEPATOCYTES, CRYOPRESERVED, NON-PLATEABLE

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 storage conditions, media, cultureware and 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.

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.

INTRODUCTION

Primary human hepatocytes are isolated from whole liver tissue obtained via the gift of organ donation from livers not suitable for organ transplant. Each donor has confirmed documentation on file allowing for non-clinical research use of any non-transplantable organs or tissues in compliance with ethical regulations. The procedures for processing the samples are Standard Operating Procedure (SOP) managed GLP protocols. All samples are collected and processed in the United States.

Human hepatocyte viability depends greatly on the use of suitable media, reagents, and recommended sterile plastic wear. If these parameters are not carefully observed cell responsiveness in assays may be lower than expected.

Primary human hepatocytes cannot be expanded or re-frozen for future uses. This cell product is for single use only.

QUALITY CONTROL (QC)

ZenBio Primary Human Hepatocytes are assessed post-thawing for both viability, number of viable cells per vial and plateability (the ability to attach to collagen I coated cultureware). Non-plateable lots are considered to be a suspension culture.
Data is reported for the viability, number of cells per vial and plateability of each lot.

Cat#	Description	Viability	Plateability (attachment)
HP-F	Cryopreserved Human Hepatocytes, Plateable, priced per million cells	>70%	>70%
HP-NP	Cryopreserved Human Hepatocytes, Non-Plateable Tier 2, priced per million cells	varies	≤50%

Each lot is tested for reactivity to viral DNA from Hepatitis B and viral RNA from HIV 1, HIV-2 and Hepatitis C by US Food and Drug Administration (FDA) licensed tests. Hepatitis B Surface antigen (HBsAg) and HIV antibody (Ab), STS (Syphilis) are also tested via by US Food and Drug Administration (FDA) licensed tests. Some lots may also be additionally tested for Cytomegalovirus (CMV) and Epstein Barr virus (EBV). However, no known test can offer complete assurance these viruses are not present. Since we cannot test all potential pathogens, please treat the culture as a potential infectious reagent. 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 at Biosafety Level 1 (BSL-1) or higher.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

- **Cryopreserved Human Hepatocytes**
  - Cat # HP-F, Cryopreserved Human Hepatocytes, Plateable
  - Cat # HP-NP, Cryopreserved Human Hepatocytes, Non-plateable
- The viable cell number per vial will vary .Contact ZenBio for the current inventory
- Store in vapor phase liquid nitrogen immediately upon receipt. Any other storage negates the warranty.
- Total transit time for human primary hepatocytes using dry ice is limited to 2-3 days only.
- Optimal transit and storage temperature is liquid nitrogen (-158°C to -190°C).

MEDIA COMPOSTIONS

Hepatocyte Plating Medium (catalog # HM-1, HM-1-250)	**Storage and Expiration Date**
DMEM (high glucose, phenol red free) Fetal Bovine Serum (FBS) Insulin, recombinant human Dexamethasone 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, 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.

Hepatocyte Maintenance Medium (catalog# HM-2, HM-2-250)	**Storage and Expiration Date**
Williams E Media (phenol red free) Insulin, recombinant human Human transferrin Sodium Selenite Bovine Serum Albumin (BSA) Linoleic acid Dexamethasone L-alanyl-L-glutamine, dipeptide Penicillin Streptomycin Amphotericin B	Store at 4°C The expiration date is 30 days from the ship date. Medium is provided ready to use and prepared fresh prior to shipment.

Primary human hepatocytes cannot be expanded or re-frozen for future uses.

This cell product is for single use only.

THAWING AND PLATING CRYOPRESERVED HUMAN HEPATOCYTES

- ❖ Thawed primary hepatocyte cells are fragile. Handle gently and work quickly to maintain viability.
- ❖ **Zen-Bio recommends the use of ZenBio brand Collagen I Coated cultureware See FAQ for details**

1. Cryovials should always be stored in vapor phase liquid nitrogen immediately upon arrival.
2. Remove the medium from the packaging material and place on ice or at 4°C. If you have media previously prepared or ordered, keep it on ice until ready to thaw the cells.
3. Remove vial of cells from liquid nitrogen and place immediately into a 37° C water bath and gently agitate while in bath. Be careful not to submerge the cap of the vial into water. Remove the vials from water bath after most of the content has thawed. Rinse the vials with 70% ethanol before taking them to the culture hood.
4. Upon thawing, and for a single cryovial, transfer the cells into a sterile 15 mL conical bottom centrifuge tube at the suggested percoll gradient mixture; see certificate of analysis.

Percoll Gradient	COLD Hepatocyte Plating Medium (cat # HM-1) with cells	Percoll	10X PBS
0%	15 mL	0	0
25%	11.25 mL	3.375 mL	0.375 mL
30%	10.5 mL	4.05 mL	0.45 mL
35%	9.75 mL	4.725 mL	0.525 mL

5. Centrifuge at 100 x g/4ºC/10 minutes.
6. Gently resuspend the cell pellet in a small volume of **COLD** Hepatocyte Plating Medium.
7. Perform a cell count using trypan blue and a hemacytometer.
8. Warm the HM-1 Medium to 37ºC prior to plating cells.
9. After counting, resuspend the cells to 750,000 cells/ml into **WARM** Hepatocyte Plating Medium.
10. Plate the cells on collagen I coated culture ware according to the guidelines in Table 1.

Note, Zen-Bio recommends the use of ZenBio, Inc. brand cultureware. See FAQ section for ordering details.

Table 1. Seeding Densities using multi-well plates coated with type I collagen

Format	Number Viable cells/ml	Volume/ well	Total # cells per well	Total volume per plate
6- well plate	750,000	2.0 ml	1.50 X 10^6	12 mL
12-well plate	750,000	1.0 ml	750,000	12 mL
24-well plate	750,000	0.5 ml	375,000	12 mL
96-well plate	750,000	125 µl	94,000	12 mL

11. Place the plates in a 37°C, 5% CO_2, humidified incubator to allow the cells to attach for 6-8 hours.
12. Observe the cells for adherence. If adherence is not complete, place the cells back in the incubator for a few hours. Once the cells are attached, aspirate the plating medium from the cells and replace with warm Hepatocyte Maintenance Medium (HM-2).

FREQUENTLY ASKED QUESTIONS

Can I pass the cells?	No. Primary human hepatocytes cannot be expanded or re-frozen for future uses. This cell product is for single use only.
I don't need all the cells in the vial. Can I use part of the vial and re-freeze the rest for future use?	No. Primary human hepatocytes cannot be expanded or re-frozen for future uses. This cell product is for single use only.
Should antibiotics be included in the medium?	Yes. Antibiotics and anti-fungal agents are always recommended since the cells are primary cells.
Where are the cells obtained?	Primary human hepatocytes are isolated from whole liver tissue obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation
Do you test for pathogens? Which ones?	Yes. Samples from each donor are tested for HIV-1, HIV-2, hepatitis B, hepatitis C and Syphilis. Some lots are additionally tested for Cytomegalovirus (CMV) and Epstein Barr Virus (EBV). However, since we cannot test all pathogens, please treat the culture as a potentially infectious agent.
What is the concentration of ingredients in your media?	We do not disclose the concentrations of the components of our media. We are happy to prepare custom media to your specifications. Please inquire.

What formats are available for the ZenBio collagen coated cultureware?	Read the Table 2 below.

Table 2. ZenBio Collagen I Coated Cultureware

Cat#	ZenBio Collagen I Coated Cultureware Description
CC-96	Collagen I Coated 96-well Plate, Pack of 5
CC-48	Collagen I Coated 48-well Plate, Pack of 5
CC-24	Collagen I Coated 24-well Plate, Pack of 5
CC-12	Collagen I Coated 12-well Plate, Pack of 5
CC-6	Collagen I Coated 6-well Plate, Pack of 5
CC-25	Collagen I Coated T-25 Flask, Vent Cap, Pack of 5
CC-75	Collagen I Coated T-75 Flask, Vent Cap, Pack of 5
CC-225	Collagen I Coated T-225 Flask, Vent Cap, Pack of 1 (EXCLUSIVE!)

PATHOGEN TESTING

Each lot is tested and found non-reactive to viral DNA from Hepatitis B and viral RNA from HIV 1, HIV-2 and Hepatitis C by US Food and Drug Administration (FDA) licensed tests. Hepatitis B Surface antigen (HBsAg) and HIV antibody (Ab), STS (Syphilis) are also tested via by US Food and Drug Administration (FDA) licensed tests. Some lots may also be additionally tested for Cytomegalovirus (CMV) and Epstein Barr virus (EBV). However, no known test can offer complete assurance these viruses are not present. Since we cannot test all potential pathogens, please treat the culture as a potential infectious reagent. 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 at Biosafety Level 1 (BSL-1) or higher.