



Human Hepatocyte Care Manual

INSTRUCTION MANUAL ZBM0003.10

SHIPPING CONDITIONS

Human Hepatocytes, Cryopreserved

Orders are delivered via Federal Express courier. All USA and Canada orders are shipped via Federal Express Priority service and are usually received the next day. Non North American International orders are usually received in 2-4 days. Primary human cells can be sensitive to extended times at dry ice temperatures. If your transit time will exceed 3 days, please inquire about dry vapor shipper options. Please inquire if alternate couriers are needed.

All orders should be processed immediately upon shipment receipt.

STORAGE CONDITIONS

HM-1 Medium: Store at +4° Expires 30 days from ship date.
Store at -20°C Expires 6 months from ship date.

HM-2 Medium: Store at +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.

Any other use negates the warranty.

All Zen-Bio Inc products are for research uses only. Not approved for human or veterinary use or for use in diagnostic or clinical procedures or other uses in humans.

ORDERING INFORMATION AND TECHNICAL SERVICES

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THIS MANUAL IS SUITABLE FOR USE WITH THE FOLLOWING PRODUCTS:

HP-F	CRYOPRESERVED PLATEABLE HUMAN HEPATOCYTES, MINIMUM OF 3 MILLION CELLS/VIAL
HP-NP	CRYOPRESERVED MATURE HUMAN HEPATOCYTES, NON-PLATEABLE, 1 MILLION CELLS/VIAL

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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 the performance of cells only if Zen-Bio media are used and the recommended storage conditions and protocols are followed without amendment or substitution. ZenBio, Inc. cryopreserved cells are assured to be viable when stored as recommended and thawed according to Zen-Bio protocols and using the recommended protocol.

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

INTRODUCTION

Primary human hepatocytes are isolated from whole liver tissue not suitable for organ transplant obtained via the gift of organ donation. 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) protocols. All samples are collected and processed in the United States.

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

QUALITY CONTROL

ZenBio Primary Human Hepatocytes are assessed post-thaw 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 PLATEABLE Human Hepatocytes, minimum of 3 million cells/vial	> 70%	> 70%
HP-NP	Cryopreserved Mature Human Hepatocytes, NON-PLATEABLE, 1 million cells/vial	varies	≤ 50%

Our QC criteria do not include tests for urea, albumin, or spheroid formation capabilities.

CATALOG ITEMS

❖ Hepatocyte Plating Medium

- Cat # HM-1 (500mL), HM-1-250 (250mL)
- Store according to label

❖ Hepatocyte Maintenance Medium

- Cat # HM-2 (500mL), HM-2-250 (250mL)
- Store 6°C to 8°C **DO NOT FREEZE**

❖ Cryopreserved Human Hepatocytes, Plateable

- Cat # HP-F
- Cryopreserved vial containing a minimum of 3 million Human Hepatocytes per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt) any other storage negates the warranty**

❖ Cryopreserved Human Hepatocytes, Non-Plateable

- Cat # HP-NP
- Cryopreserved vial containing 1 million Human Hepatocytes per vial **(store in vapor phase liquid nitrogen IMMEDIATELY upon receipt) any other storage negates the warranty**

MEDIA COMPOSTIONS

<u>Hepatocyte Plating Medium</u> (catalog # HM-1, HM-1-250)	<u>Storage and Expiration Date</u>
DMEM/ Ham's F-12 (1:1, v/v), 3.15 g/L (17.5 mmol/L) D-glucose (phenol red free) Fetal Bovine Serum (FBS; USA Origin) Human Insulin, recombinant Dexamethasone Penicillin Streptomycin Amphotericin B	<ul style="list-style-type: none"> • If stored at +4°C upon arrival, the media is stable until the expiration date on the bottle. • If stored at -20°C upon arrival, the media is stable for 6 months. <i>The media will expire 30 days after the thaw date.</i> • Medium is provided ready to use and prepared fresh prior to shipment.

<u>Hepatocyte Maintenance Medium</u> (catalog # HM-2, HM-2-250)	<u>Storage and Expiration Date</u>
William's E Medium (phenol red free) Human Insulin, recombinant Human apo-Transferrin Sodium Selenite Bovine Serum Albumin (BSA) Linoleic acid Dexamethasone L-alanyl-l-glutamine (dipeptide glutamine) Penicillin Streptomycin Amphotericin B	<ul style="list-style-type: none"> • 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. <p style="text-align: center;"><u>DO NOT FREEZE</u></p>

There is no cryopreservation medium provided as primary human hepatocytes cannot be expanded or re-frozen for future uses.

This cell product is for single use only.

THAWING CRYOPRESERVED CELLS

PRIMARY HUMAN HEPATOCYTES

Zen-Bio recommends the use of ZenBio brand Collagen I Coated cultureware See FAQ for details

Note: Thawed primary hepatocyte cells are fragile. Handle gently and work quickly to maintain viability.

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, no more than 1 minute. 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 / 4°C for 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 37°C 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 per mL	Volume per Well	Total # Cells per Well	Total Volume per Plate
6- well plate	750,000	2.0 mL	1,500,000	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% CO₂, 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 (cat # HM-2).

FREQUENTLY ASKED QUESTIONS

Can I passage the cells?

No. Primary human hepatocytes cannot be expanded or re-frozen for future uses. This cell product is for single thaw and 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 thaw and use only.

Should antibiotics be included in the medium?

Yes. Antibiotics and anti-fungal agents are always recommended for 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. Each lot of primary cells is tested via PCR and found non-reactive to viral DNA from HIV and hepatitis B and viral RNA from Hepatitis C. However, no known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially 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.

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 for custom formulations.

My cells are not secreting albumin or urea and do not test positive for spheroids. Is there something wrong with my cells?

Our QC criteria do not include tests for urea, albumin, or spheroid formation capabilities.

Is there a specific type of cultureware that should be used?

Yes, only Zen-Bio Collagen Coated Cultureware should be used (see Table below).

Item#	Item Description
CC-6	Collagen I Coated 6-well Plate, Pack of 5
CC-12	Collagen I Coated 12-well Plate, Pack of 5
CC-24	Collagen I Coated 24-well Plate, Pack of 5
CC-48	Collagen I Coated 48-well Plate, Pack of 5
CC-96	Collagen I Coated 96-well Plate, Pack of 5
CC-384	Collagen I Coated 384-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-175	Collagen I Coated T-175 Flask, Vent Cap, Pack of 5
CC-225	Collagen I Coated T-225 Flask, Vent Cap, Pack of 1 (EXCLUSIVE!)

PATHOGEN TESTING

Samples from each donor are tested via PCR and found non-reactive to viral DNA from HIV and hepatitis B, viral RNA from Hepatitis C and Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) via FDA licensed tests. Some samples may display reactivity to common viruses Epstein Barr Virus (EBV) or Cytomegalovirus (CMV). Samples that test reactive to CMV or EBV indicate infection at some point in life. No known test can offer complete assurance that these viruses are not present. Since we cannot test all pathogens, always treat the culture as a potentially 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 or higher.