



Human Hepatocytes Care Manual

INSTRUCTION MANUAL ZBM0003.07

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 transit time will exceed 3 days. Hepatic cells are very 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. **Must be processed immediately upon shipment receipt.**

STORAGE CONDITIONS

Media: Store as indicated IMMEDIATELY UPON ARRIVAL

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-NP2, HP-NP3, HP-NP4	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 the performance of cells only if Zen-Bio media are used and the recommended protocols are followed. Cryopreserved cells are assured to be viable when thawed according to Zen-Bio protocols and using the recommended cultureware.

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

MATERIALS PROVIDED FOR EACH CATALOG ITEM

❖ Cryopreserved Human Hepatocytes

- Cat # HP-F, HP-NP2, HP-NP3, HP-NP4
- Frozen vial containing primary human hepatocytes. Cell number will vary. Contact ZenBio for the current inventory
 - Store in vapor phase liquid nitrogen immediately upon receipt

MEDIA COMPOSTIONS

<u>Hepatocyte Plating Medium</u> <u>(catalog # HM-1, HM-1-250)</u>	<u>Storage and Expiration Date</u>
DMEM (high glucose, phenol red free) Fetal Bovine Serum (FBS) Insulin, recombinant human Dexamethasone Penicillin Streptomycin Amphotericin B	<p>If placed at 4°C upon arrival, the media is stable until the expiration date on the bottle label.</p> <p>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.</p>

<u>Hepatocyte Maintenance Medium</u> <u>(catalog# HM-2, HM-2-250)</u>	<u>Storage and Expiration Date</u>
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	<p>Store at 4°C</p> <p>The expiration date is 30 days from the ship date.</p> <p>Medium is provided ready to use and prepared fresh prior to shipment.</p>

THAWING AND PLATING CRYOPRESERVED HEPATOCYTES

NOTE: THAWED CELLS ARE FRAGILE. HANDLE GENTLY AND QUICKLY TO MAINTAIN VIABILITY.

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

1. Cryovials should be stored in 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	<u>COLD</u> 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/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 medium to 37°C prior to plating cells.
9. After counting, resuspend the cells to 7.5 X 10⁵ 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 BioCoat® brand cultureware from Becton-Dickinson.

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	7.5 X 10 ⁵	2.0 ml	1.50 X 10 ⁶	12 mL
12-well plate	7.5 X 10 ⁵	1.0 ml	7.50 X 10 ⁵	12 mL
24-well plate	7.5 X 10 ⁵	0.5 ml	3.75 X 10 ⁵	12 mL
96-well plate	7.5 X 10 ⁵	125 µl	9.4 X 10 ⁴	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 (HM-2).

FREQUENTLY ASKED QUESTIONS

Can I pass the cells?	No. Primary human hepatocytes are a thaw and use one time only product
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 via PCR to confirm non-reactivity for HIV-1, HIV-2, hepatitis B, hepatitis C and syphilis. 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.
What formats are available for the ZenBio collagen coated cultureware?	See the chart below.

ZenBio Collagen I Coated Cultureware

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