



RODENT Hepatocytes Care Manual

INSTRUCTION MANUAL ZBM0054.02

SHIPPING CONDITIONS

Rodent Hepatocytes cryopreserved

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

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:

HPR-F	CRYOPRESERVED RAT (SD OR WISTAR) HEPATOCYTES, PLATEABLE
HPR-NP	CRYOPRESERVED RAT (SD OR WISTAR) HEPATOCYTES, NON- PLATEABLE
HPM-F	CRYOPRESERVED MOUSE (CD-1) HEPATOCYTES, PLATEABLE.
HPM-NP	CRYOPRESERVED MOUSE (CD-1) HEPATOCYTES, NON-PLATEABLE.

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.

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. Rodent 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.

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.

MATERIALS PROVIDED FOR EACH CATALOG ITEM

❖ Cryopreserved Mouse Hepatocytes

- Cat# HPM-F, HPM-NP
- Frozen vial containing primary mouse hepatocytes.
- Cell number: \geq 4 million cells/vial.
- Contact ZenBio for the current inventory
- Store in vapor phase liquid nitrogen immediately upon receipt

❖ Cryopreserved Rat Hepatocytes

- Cat # HPR-F, HPR-NP,
- Frozen vial containing primary rat hepatocytes.
- Cell number: \geq 4 million cells/vial.
- Contact ZenBio for the current inventory
- Store in vapor phase liquid nitrogen immediately upon receipt

MEDIA COMPOSTIONS

<u>Rodent Hepatocyte Plating Medium</u> <u>(catalog # HM-3)</u>	<u>Storage and Expiration Date</u>
Williams E Medium (phenol red free) HEPES Fetal Bovine Serum (FBS) Insulin Dexamethasone L-alanyl-L-glutamine (dipeptide glutamine) Gentamicin	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.
<u>Rodent Hepatocyte Maintenance Medium</u> <u>(catalog# HM-4)</u>	<u>Storage and Expiration Date</u>
Williams E Medium (phenol red free) HEPES Insulin Transferrin Sodium Selenite Linoleic acid Dexamethasone L-alanyl-L-glutamine (dipeptide glutamine) Gentamicin	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 3 months . Add fresh antibiotics when you are ready to use. Add fresh antibiotics when you are ready to use. The media will expire 30 days after the thaw date.

THAWING AND PLATING CRYOPRESERVED RODENT HEPATOCYTES

NOTE: THAWED HEPATOCYTES 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**

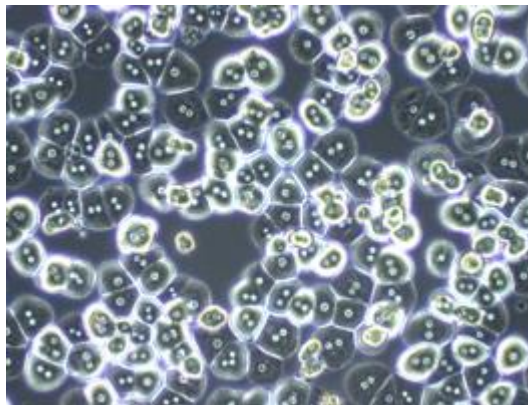
1. Cryovials should be stored in vapor phase liquid nitrogen immediately upon arrival.
2. Remove the medium from the packaging material and let it come to ambient temperature. Remove vial of cells from liquid nitrogen and place immediately into a 37° C water bath and gently agitate while in bath. Be careful not to submerge the cap of the vial into water. Remove the vials from water bath after most of the content has thawed. This process should take no more than 2 minutes. Rinse the vials with 70% ethanol before taking them to the culture hood.
3. **DO NOT** USE A PIPET TO DISPENSE THE CELLS FROM THE VIAL. Gently pour the contents of the vial into a 50ml sterile conical bottom centrifuge tube containing 30-35ml of ambient temperature Hepatocyte Plating Medium (cat # HM-3).
4. Rinse the vial by gently POURING 1-2ml HM-3 into the vial and gently POUR the contents to the same 50ml centrifuge tube.
5. Centrifuge at 50 X g for 3 minutes, 20°C.
6. Gently aspirate all of the supernatant while being careful not to disturb the cell pellet. Add media by pouring along the side of the tube, not directly onto the pellet. Add 5mls of ambient temperature media to the tube. Gently mix the sealed tube using your hands like a seesaw to gently rock the tube until no clumps remain. It is vital that you resuspend the pellet using this gentle rocking motion and DO NOT INVERT the tube!
7. Perform a cell count using the trypan blue exclusion method and a hemacytometer. A 1:2 or 1:5 dilutions is recommended.
8. Adjust cellular density to the desired concentration for your application using ambient temperature plating medium HM-3. You may need to visually assess the seeding density for optimal results; however, it is IMPORTANT to note that over-seeding rodent hepatocytes can lead to cell death.

9. See the recommended concentration of cells needed to seed in collagen I cultureware.
- **Mouse Hepatocytes:** Dilute your cell suspension to have a concentration of 400,000 cells/ml.
 - **Rat Hepatocytes:** Dilute your cell suspension to have a concentration of 700,000 cells/ml.
 - Approximately 12 ml Plating Medium will be required per plate for multi-well plates. See individual well volumes in the table below.

Table 1. Volumes required for using multi-well plates coated with type I collagen

Format	Volume media per well	Total Volume media per plate
96 well plate	0.125 ml	12.0 ml
24 well plate	0.5 ml	12.0 ml
12 well plate	1.0 ml	12.0 ml
6 well plate	2.0 ml	12.0 ml

10. Add the cell suspension to your plate. Plate the cells on collagen coated culture ware according to the guidelines in Table 1.
11. Place the plates in a 37°C, 5% CO₂, humidified incubator. Hold the plate flat on the incubator shelf and gently shake it in a left to right, then forward to back pattern to evenly disperse the cells throughout the plating surface. Avoid circular motions that will result in the piling up of cells. Allow the cells to attach for 4-5 hours minimum.



12. Once the cells have sufficiently attached, carefully aspirate the plating medium and replace with warm (37° C) maintenance media (HM-4). **IMPORTANT—Pipette the new media into the well along the side of the well—DO NOT pipette media directly into the newly formed monolayer.**

13. If your application requires a sandwich culture, you may perform a Matrigel overlay after the culture is established.

A. Mouse Hepatocytes

1. Aspirate the maintenance medium (HM-4) and replace with cold HM-4 medium (containing Matrigel®) 6-8 hours after the first media change (8-9 hours after initial seeding).
2. Replace media every 24 hours thereafter with warm (37⁰ C) maintenance media.

B. Rat Hepatocytes

1. Aspirate the maintenance medium and replace with cold H-4 medium (containing Matrigel®) 3-4 hours after the first media change (11-13 hours after initial seeding).
2. Replace media every 24 hours thereafter with warm (37⁰ C) maintenance media HM-4.

FREQUENTLY ASKED QUESTIONS

1	Can I expand the cells to a higher passage?	No. The cells may only be used once upon thawing.
2	How long can the cells remain in culture?	Cells may remain in culture 3-5 days. After 3 days, cells will begin to decline in viability and hepatic functions.
3	What happens if I use a serological pipet to transfer the cells from the vial to my centrifuge tube?	Rodent hepatocytes are very fragile. Using a pipet or other repeated mechanical handling may result in the loss of viable cells. We recommend using only the methods recommended for thawing and handling rodent hepatocyte cells in this manual.
4	Do you test for pathogens?	No. All rats and mice are sourced from Specified Pathogen Free (SPF) colonies.
5	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.
6	What formats are available for the ZenBio collagen coated cultureware?	See the Table 2.
7	How many cells are in each well in a multi-well plate?	See Table 3.

Table 2. 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

Table 3. Cell density per well

Format	Volume media per well	MOUSE Hepatocytes 400,000 cells/ml # cells/well	Rat Hepatocytes 700,000 cells/ml # cells/well
96 well plate	0.125 ml	50,000	87,500
24 well plate	0.5 ml	200,000	350,000
12 well plate	2.0 ml	400,000	700,000
6 well plate	2.0 ml	800,000	1.4 x 10 ⁶