



Human Hepatocytes Care Manual

INSTRUCTION MANUAL ZBM0003.05

SHIPPING CONDITIONS

Human Hepatocytes cryopreserved

Orders are delivered via Federal Express courier.

Must be processed immediately upon shipment receipt.

STORAGE CONDITIONS

Media: Store at 2-8°C

Cryopreserved cells: Liquid nitrogen

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.

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 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.

ORDERING INFORMATION AND TECHNICAL SERVICES

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MEDIA COMPOSTIONS

Hepatocyte Plating Medium (catalog # HM-1)

DMEM (high glucose, phenol red free)
Fetal Bovine Serum (FBS)
Insulin, recombinant human
Dexamethasone
Penicillin
Streptomycin
Amphotericin B

Hepatocyte Maintenance Medium (catalog# HM-2)

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

NOTE:

All media are provided ready to use and prepared fresh prior to shipment.

The expiration date of all media is 30 days from the ship date.

Please schedule your orders accordingly.

THAWING AND PLATING CRYOPRESERVED HEPATOCYTES

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

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 15ml 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 media to 37°C prior to plating.
9. After counting, resuspend the cells to 7.5×10^5 cells/ml into warm Hepatocyte Plating Medium.
10. Plate the cells on collagen 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 or Matrigel

Format	Number Viable cells/ml	Volume/well	Total # cells per well	Total volume per plate
6- well plate	7.5×10^5	2.0 ml	1.50×10^6	12 ml
12-well plate	7.5×10^5	1.0 ml	7.50×10^5	12 ml
24-well plate	7.5×10^5	0.5 ml	3.75×10^5	12 ml
96-well plate	7.5×10^5	125 μ l	9.4×10^4	12 ml

10. Place the plates in a 37°C, 5% CO₂, humidified incubator to allow the cells to attach for 6-8 hours.
11. 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).