HUMAN HEPATOCYTES
INSTRUCTION MANUAL
ZBM-3

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

For in Vitro Use Only

PRECAUTIONARY NOTES

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

Zen-Bio, Inc.
3200 Chapel Hill-Nelson Blvd., Suite 104
PO Box 13888
Research Triangle Park, NC 27709
U.S.A.

Telephone	(919) 547-0692	Toll free	1-(866)-234-7673
Facsimile (FAX)	(919) 547-0693		
Electronic mail (e-mail)	information@zen-bio.com		
World Wide Web	http://www.zen-bio.co		

MEDIA COMPOSITIONS

Hepatocyte Plating Medium (catalog # HM-1)

RPMI-1640
Fetal Bovine Serum (FBS)
L-alanyl-L-glutamine (dipeptide glutamine)
Insulin, from bovine pancreas
Human transferrin
Sodium Selenite
Pyruvate
Ethanolamine
2-mercaptoethanol
Bovine serum albumin, lipid rich
Nicotinamide
Penicillin
Streptomycin

Hepatocyte Maintenance Medium (catalog# HM-2)

RPMI-1640
L-alanyl-L-glutamine (dipeptide glutamine)
Insulin, from bovine pancreas
Human transferrin
Sodium Selenite
Pyruvate
Ethanolamine
2-mercaptoethanol
Bovine serum albumin, lipid rich
Nicotinamide
Penicillin
Streptomycin

NOTE:
All media are provided ready to use.
All media are also available as phenol red free. Please inquire.

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, transfer the cells to a sterile conical bottom centrifuge tube containing 15 ml of COLD Hepatocyte Plating Medium (cat # HM-1). Place the tube on ice. Rinse the vial using 1-2 ml medium and add the contents to the same tube.
5. Centrifuge at 100 X g / 4°C / 5 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 X 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^R 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 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

10. Place the plates in a 37°C, 5% CO_2, 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).