

Human Hepatocyte Care Manual

INSTRUCTION MANUAL (Cryo Vial)

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 SciKon Innovation 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

MAIL / BILL (USPS): SCIKON INNOVATION, INC.

PO Box 9100

CHAPEL HILL, NC 27515

PHYSICAL (COURIER): FFVC / SCIKON INNOVATION, INC.

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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 culture medium from 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 Seeding Medium 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 110 X g / 4°C / 10 minutes.
6. Gently resuspend the cell pellet in a small volume of Hepatocyte Seeding 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 Seeding Medium.
10. Plate the cells on collagen coated culture ware according to the guidelines in Table 1.

Note, Scikon recommends the use of BioCoat® brand cultureware from Becton-Dickinson.

Table 1. Seeding Densities using multi-well plates

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 µl	9.4×10^4	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 seeding medium from the cells and replace with warm Hepatocyte Maintenance Medium.

MEDIA COMPOSTIONS

<u>Hepatocyte Seeding Medium</u>	<u>Hepatocyte Maintenance Medium</u>	<u>NOTE:</u>
DMEM (high glucose, phenol red free) Fetal Bovine Serum (FBS) Insulin Glutamax I Dexamethasone Antibiotic / Antimycotic	Williams E Media (phenol red free) Insulin Transferrin Sodium Selenite Dexamethasone Glutamax I Antibiotic / Antimycotic	<p>All media are provided ready to use and prepared fresh prior to shipment.</p> <p>The expiration date of all media is 30 days from the ship date.</p> <p>Please schedule your orders accordingly.</p>

– end Human Hepatocyte Care Manual