

Mouse Hepatocyte Care Manual

INSTRUCTION MANUAL (Cryo Vial)

SHIPPING CONDITIONS

Rat 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 animal origin, due to their potential biohazardous nature. **Always wear gloves and work behind a protective screen when handling primary animal cells.** All media, supplements, and tissue culture ware used in this protocol should be sterile.

Mouse 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.

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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.

Preparation:

- Have all media completed and ready to use: ~35 mls of room-temp media per cryo vial.
- Have Trypan Blue solution prepared for cell viability and counting.
- Have the cell culture vessel readily available for seeding.

Thaw Process:

1. Remove the vial from the storage freezer and immediately transfer to a 37° C water bath.
2. Place cryo vial in 37° C water, but do not completely submerge the vial. Be careful to keep the cap above the water. Gently shake to achieve even thawing. Remove the vial when contents have thawed into an “ice slurry” suspension (generally less than 2 minutes).
3. Once thawed, wipe off outside of the vial with 70% ethanol.
4. Upon thawing, and for a single cryo vial, transfer the cells into a sterile 15ml conical bottom centrifuge tube at the suggested percoll gradient mixture; see certificate of analysis.

Process	Percoll Gradient	<u>COLD</u> Hepatocyte Seeding Medium with cells	Percoll	10x PBS
i	0%	15 ml	0	0
ii	15%	12.75 ml	2.025 ml	0.225 ml
iii	20%	12 ml	2.7 ml	0.3 ml
iv	25%	11.25 ml	3.375 ml	0.375 ml
v	30%	10.5 ml	4.05 ml	0.45 ml
vi	35%	9.75 ml	4.725 ml	0.525 ml

5. Gently mix cells by a rocking motion.
6. For process (i), go directly to instruction 7. For process (ii – vi), centrifuge at 110 x g / 4°C/ 10 min, then go to instruction 8.
7. Centrifuge at 50 x g / 4°C/ 3 min.
8. Aspirate all of the supernatant being careful not to disturb the cell pellet.
9. Gently resuspend the cell pellet in a small volume of room-temp Hepatocyte Seeding Medium. It is best to pour along the side of the tube, and not directly onto the cell pellet.
10. Determine cell counts using the Trypan Blue exclusion method.

Cell Plating:

11. Adjust cellular density to the desired concentration for your application, using room-temp seeding media. Seeding recommendations are shown below:

Species	Cells	Recommended Seeding Density	6 well	12 well	24 well	48 well	96 well
Mouse	Hepatocytes	0.40 x 10 ⁶ cells / ml	2 ml	1 ml	0.5 ml	0.25 ml	0.125 ml
Rat	Hepatocytes	0.70 x 10 ⁶ cells / ml	2 ml	1 ml	0.5 ml	0.25 ml	0.125 ml

You may need to visually assess the seeding density for optimal results. Over-seeding rodent hepatocytes can lead to cell death. Also, under-seeding provides inferior functional results.

12. With optimal seeding concentrations, add cell suspensions to your plate (collagen-coated plates are recommended).
13. Position plate in a tissue culture incubator (37°C, 5% CO₂). Gently agitate in a figure 8 motion to evenly disperse cells. Avoid circular motions that accumulate cells at plate edges.

Cell Attachment:

14. Allow 4-5 hours for attachment. Observe the cells for adherence. If adherence is not complete, place the cells back in the incubator for a few hours. Once the cells have sufficiently attached, carefully aspirate the seeding media and replace with warm (37°C) maintenance media. Pipette the new media into the well along the side-wall of the well – do not pipette the media directly onto the newly formed cell monolayer. Replace media every 24 hours thereafter with warm maintenance (37°C) media.

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) ITS Dexamethasone Glutamax I Antibiotic / Antimycotic	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.

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