

# Human Total Liver Cell (TLC) Care Manual

**INSTRUCTION MANUAL (Cryo Vial)**

**SHIPPING CONDITIONS**

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**Human Total Liver Cells, cryopreserved**

Orders are delivered via Federal Express courier.

**Must be processed immediately upon shipment receipt.**

**STORAGE CONDITIONS**

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**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**

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**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 Total Liver Cell (TLC) 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.

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## ORDERING INFORMATION AND TECHNICAL SERVICES

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

PO Box 9100

CHAPEL HILL, NC 27515

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

2 DAVIS DRIVE, SUITE 209

RESEARCH TRIANGLE PARK, NC 27709

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## THAWING AND PLATING CRYOPRESERVED TLC POPULATIONS\_\_

NOTE: THAWED TOTAL LIVER CELLS 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> TLC Culture 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. See cell explanations below.
  - A. For 0% percoll gradient; all liver cells remain in the pellet. This is your TLC population.
  - B. For percoll gradients 25%, 30%, & 35%; TLC populations are in the cell pellet. Combined donor parameters, density gradients, and centrifugation dictate our QC outcomes.
    - i) Occasionally, and depending on donor parameters with different cell densities, media supernatants in item “B” are determined to have additional TLC populations. This information will be noted on the CofA (e.g. retain supernatant for additional cells).
6. For pelleted cells, gently resuspend the pellet in a small volume of TLC Culture Medium.
  - C. If you desire the supernatant cells, place the supernatant in a 50ml centrifuge tube, double the media volume with TLC Culture Medium. Centrifuge at 200Xg / 4°C / 5 minutes. This new (2<sup>nd</sup>) pellet contains nonparenchymal cells with some hepatocytes. Remove the supernatant. Gently resuspend pellet in a small volume of TLC Culture Medium.
7. For all pellets, perform a cell count using trypan blue.
8. Warm the TLC Culture Media to 37°C prior to plating.
9. After counting, resuspend Hepatocytes to 7.5 X 10<sup>5</sup> cells/ml into warm TLC Culture Medium.
  - D. Add nonparenchymal cells at levels that support your study.

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 Hepatocyte Densities using multi-well plates. Nonparenchymal counts are usually not included during cell seeding, as their size is significantly smaller than Hepatocytes.**

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

Add nonparenchymal cells at levels that support your study.

11. Place the plates in a 37°C, 5% CO<sub>2</sub>, 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 initial TLC Culture Medium, containing unattached cells, and replace with fresh and warm TLC Culture Medium.

## MEDIA COMPOSTION

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

– end Human TLC Care Manual