SciKon Innovation, Inc.

# Human Non-Parenchymal Care Manual

## INSTRUCTION MANUAL (Cryo Vial)

## SHIPPING CONDITIONS

**Human Non-Parenchymal Cells 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 non-parenchymal cell 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. | PHYSICAL (COURIER): FFVC / SCIKON INNOVATION, INC. |
| PO BOX 9100 | 2 DAVIS DRIVE, SUITE 209 |
| CHAPEL HILL, NC 27515 | RESEARCH TRIANGLE PARK, NC 27709 |

PHONE: 919.354.1083 FAX: 919.990.8561 EMAIL: INFO@SCIKONINNOVATION.COM

# THAWING AND PLATING CRYOPRESERVED NON-PARENCYMALS

NOTE: THAWED NON-PARENCHYMAL 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 | COLD Non-Parenchymal 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 Non-Parenchymal 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 1.5 X $10^6$ cells/ml into warm Nonparenchymal 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 X $10^5$ | 2.0 ml | 3.0 X $10^6$ | 12 ml |
| 12-well plate | 7.5 X $10^5$ | 1.0 ml | 1.50 X $10^6$ | 12 ml |
| 24-well plate | 7.5 X $10^5$ | 0.5 ml | 7.5 X $10^5$ | 12 ml |
| 96-well plate | 7.5 X $10^5$ | 125 µl | 1.88 X $10^5$ | 12 ml |

11. Place the plates in a 37°C, 5% $CO_2$, 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 non-parenchymal medium from the cells and replace with warm and fresh Non-Parenchymal Medium.

## MEDIA COMPOSTIONS

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

– end Human Non-Parenchymal Cell Care Manual