



# Human Non Parenchymal Care Manual

INSTRUCTION MANUAL ZBM0052.00

## SHIPPING CONDITIONS

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### Human Non Parenchymal 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 Zen-Bio Inc 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 nonparenchymal 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

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## MEDIA COMPOSTIONS

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### Hepatic Plating Medium (catalog # HM-1)

DMEM (high glucose, phenol red free)

Fetal Bovine Serum (FBS)

Insulin, recombinant human

Dexamethasone

Penicillin

Streptomycin

#### NOTE:

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.**

## THAWING AND PLATING CRYOPRESERVED NONPARENCHYMAL CELLS

NOTE: THAWED NONPARENCHYMALS ARE FRAGILE. HANDLE GENTLY AND QUICKLY TO MAINTAIN VIABILITY.

**ZEN-BIO RECOMMENDS THE USE OF BIOCOAT® BRAND CULTUREWARE FROM BECTON-DICKINSON.**

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 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 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  $7.5 \times 10^5$  cells/ml into warm 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® 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 'cell stock' volume per plate	Percentage of Surface Coverage
6- well plate	$7.5 \times 10^5$	1.0 ml	$7.5 \times 10^5$	6 ml	80%
6- well plate	$7.5 \times 10^5$	0.75 ml	$5.6 \times 10^5$	4.5 ml	60%
6- well plate	$7.5 \times 10^5$	0.5 ml	$3.75 \times 10^5$	3 ml	40%
6- well plate	$7.5 \times 10^5$	0.25 ml	$1.87 \times 10^5$	1.5 ml	20%

10. Place the plates in a 37°C, 5% CO<sub>2</sub>, 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 initial plating medium from the cells and replace with warm and fresh plating medium (HM-1).