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HUMAN PRIMARY
NON-PARENCHYMAL CELLS

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PRODUCT DETAILS

Tissue	Human liver
Package Size	≥1.000.000 c/vial
Shipped	Cryopreserved
Storage	Liquid Nitrogen

PROCEDURE

The aim of the present method is to describe the process of thawing human cryopreserved non-parenchymal cells.

Please read through this entire protocol before attempting this procedure.

Required and recommended reagents and consumables

- Thawing protocol
  - **MNT:** NPCs Thawing Medium

1. Arrival of the cryopreserved cells in your facilities

Store the cryogenic hepatic stellate vials immediately in vapor phase nitrogen upon receipt.

2. Thawing protocol for cryopreserved primary human non-parenchymal cells

Note: Handle gently and quickly to maintain viability.

Thawing of primary human non-parenchymal cells

1. Before starting the thawing process, switch on a refrigerated centrifuge at 4°C.
2. Remove the cryopreserved hepatic non-parenchymal cells from the liquid nitrogen and immediately place it in a 37°C water bath, hold and rotate the vial gently until the contents are partially thawed.
3. Remove the vial from the water bath, wipe dry, rinse the cryovial with 70% ethanol for disinfection and transfer it into the sterile flow hood.
4. Using a pipette, gently transfer the cell suspension contained in the cryovial into a sterile 15 mL conical tube with 10 ml cold MNT.
5. Add 1 ml of MNT to the cryovial, in order to recover the cells that have remained inside

each cryovial. Then, combine it with the cells in the 15 mL conical tube to ensure that all non-parenchymal cells have been transferred.

6. Centrifuge the tube at 650g for 10 min at 4°C.

7. After centrifugation, aspirate the supernatant and re-suspend the cell pellet in 1-3 mL of cold MNT by gently agitating the bottom of the tube.

8. Perform a cell count (see the next section: determination of viability and number of cells).

9. After determining the number of cells, proceed with the required assay.

Determination of viability and number of cells

To determine viability and number of cells from the stellate cells' suspension in step 8, please follow these instructions:

- Carefully homogenize the cell suspension (do not vortex).
- Take 20 µl of the cell suspension and mix it in a 1.5 ml tube with 20 µl of PBS, and 20 µl of Trypan Blue solution (1/3 dilution) and keep the rest of the cell suspension on ice in the laminar flow hood until seeding.
- Gently homogenize the solution by pipetting up/down.
- Add an aliquot of 20 µl with the Trypan Blue/cell mixture into a cell counting chamber.
- Proceed with cell observation and counting under the microscope. Dead cells will appear in blue. In order to determine viability and cell number, count the living and dead cells and use these formulas:

Viability (%) = (Live cell count/(Live cell count + dead cell count)) x 100

Total Cell number = (Viable cell count/Quadrants counted) x Dilution factor x 10^4* x Current volume(mL)

**This factor is applicable when it is used a Hemocytometer*

***To keep in mind: During cell counting, the identification of cell debris and hepatocytes (bigger cells) is probable. Please do not consider these cells as part of the cell counting. NPCs should be identified as the smallest and brightest cells.**