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Thawing and Plating Cryopreserved Non-Parenchymal Cells

This protocol is suitable for the handling of cryopreserved Non-Parenchymal cells. Please read through this entire protocol before attempting this procedure. The health of the cells is dependent upon following the protocol carefully.

Procedure for Thawing and Plating Cryopreserved Non-Parenchymal Cells

Note: Handle gently and quickly to maintain viability. Collagen I coated culture ware is required. DO NOT pre-warm medium to thaw cells.

1. Place vial in a 37°C water bath, hold and rotate vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe dry, rinse the vial with 70% ethanol and transfer to a biological safety cabinet. Remove cap, being careful not to touch the interior threads with fingers.
2. Using a pipette, gently transfer contents of vial to a conical tube containing 15 mL of cold (4°C) Plating Medium (MP100) and place the tube on ice. Rinse the vial using 1-2 ml medium and add the contents to the same tube.

   Note: Nonparenchymal cells easily attach to the walls of the conical tube at 37°C. Therefore, use of pre-warmed media is not recommended at this step.
3. Centrifuge tube at 200xg for 5 minutes in 4°C. After centrifugation, aspirate medium and re-suspend cell pellet in 1mL cold Plating Medium (MP100)
4. Count the cells using the Trypan Blue Exclusion Assay.
5. Dilute the cells in warm Plating Medium (MP100) to 750,000 cells/mL.
6. Plate the cells on culture ware coated with collagen type I, see Table 1 for seeding densities.

Table 1.

Well Format	Volume per well (mL)	# Cells Per Well	Total Volume per Plate (mL)
6	2.0	1,500,000	12
12	1.0	750,000	12
24	0.5	375,000	12
96	0.125	94,000	12

7. Place the cells in a humidified 37°C/5% CO_2 incubator and allow them to attach for 6-8 hours.
8. Assess attachment after initial time period. If adherence is not complete, place the cells back in the incubator for another 3-4 hours. If attachment has already occurred, move on to step 9.
9. After attachment, replace the medium with fresh warm Plating Medium (MP100).