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Triangle Research Labs, LLC | www.TRLcells.com
U.S. Scientific Support: 800 521 0390 | scientific.support@lonza.com
EU/ROW Scientific Support: +32 87 321 611 | scientific.support.eu@lonza.com

Thawing & Plating Cryopreserved Human Hepatic Stellate Cells

This protocol is suitable for the handling of cryopreserved Stellate 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 Stellate Cells

Note: Handle gently and quickly to maintain viability. Collagen I coated culture ware is recommended.

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 sterile work area. Remove cap, being careful not to touch the interior threads with fingers.
2. Using a pipette, gently transfer contents of vial to a sterile 15 mL conical tube.
3. Wash vial with 5 mL MCST250 medium and add this wash to conical tube.
4. Centrifuge the tube at 250*g* for 5 minutes. After centrifugation, aspirate medium and re-suspend the contents in fresh MCST250 medium.
5. Count the cells using the Trypan Blue Exclusion Assay.
6. For expansion, seed the cells at a density of 4,000 cells/cm^2 on collagen I coated plates.
7. For best results, do not disturb the culture for at least 12 hours after seeding. Change medium the next day to remove any residual DMSO or unattached cells, then every other day thereafter.

Instructions for Sub-Culturing Stellate Cells

1. Subculture cells when they have reached 90% confluency.
2. Warm medium, 0.25% trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS) to room temperature.
3. Aspirate medium, then rinse cells with DPBS. Add trypsin solution into flask and incubate in a 37˚C incubator for 3-5 minutes, or until the cells detach.
4. At the end of typsinization, wash cells off flask with an appropriate amount of medium.
5. Transfer to centrifuge tube and centrifuge at 250*g* for 5 minutes.
6. After centrifugation, aspirate the medium, re-suspend in 1-2 mL fresh medium and count cells for seeding.
7. Seed the cells at a density of 4,000 cells/cm^2 on collagen I coated plates.

Frequently Asked Questions

1. How far can I expand the Stellate cells?
   Human primary stellate cells have no confirmed limit for expansion. We recommend terminal passage at passage 5. Cells are cryopreserved after establishing viable cultures.
2. Can I co-culture the Stellate cells with hepatocytes?
   Yes, the recommended ratio is 2 hepatocytes to 1 Stellate cell. Stellate cells are plated first. Hepatocytes are seeded after initial Stellate cell attachment.

Example Images