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Culturing Cryopreserved Stellate Cells
Instructions for Use

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I. Introduction

Stellate cells are a resident cell type of the liver primarily functioning to store retinoids. In response to liver damage, Stellates rapidly lose the stored retinoids and differentiate into a proliferating fibroblast-like cell that begins depositing collagen matrix. This activity causes buildup of collagen in the liver eventually leading to cirrhosis. Stellate cells can be isolated from disrupted liver tissue, enriched, and placed into cell culture.

For answers to Frequently Asked Questions regarding these products, please visit our FAQ Database:

www.lonza.com/faq

For references citing the use of these products, please visit our Citation Database:

www.lonza.com/citations

II. Required Reagents and Materials

(Components Sold Separately)

1. Cryopreserved Stellate Cells
2. Stellate Cell Growth Medium (Lonza catalog number MCST250)
3. 0.25% trypsin solution(Lonza catalog number 17-161E or equivalent)
4. Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (Lonza catalog number 17-512F or equivalent)
5. 0.4%Trypan Blue solution (Lonza catalog number 17-942E or equivalent)
6. Collagen coated cell culture plates (e.g. Corning™ BioCoat™ Collagen I Multiwell Plates or equivalent)

NOTE: Using media or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

III. General Cell Information

Cat. No.	Product Name	Description	Size
HUCLS	Human Stellate Cells - Passage 1	Cryovial	≥100,000 cells/vial
HUCLS1	Human Stellate Cells - Passage 1	Cryovial	≥ 1,000,000 cells/vial
HUCLS1-P0	Human Stellate Cells - Passage 0	Cryovial	≥ 1,000,000 cells/vial
MCST250	Human Stellate Growth Medium	Bottle	250 mL

IV. Unpacking and Storage Instructions

1. For cryopreserved cells: Remove cryovials from the liquid nitrogen shipping dewar or dry ice container and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If shipping container is warm, please contact Customer Service.
2. Store Stellate Cell Growth Medium at 2°-8°C.

V. Preparation of Culture Medium

1. Warm medium in a 37°C water bath prior to thawing cryopreserved cells.
2. Decontaminate external surfaces of all vials and the medium bottle with ethanol or isopropanol.

VI. Thawing and Plating Cells

NOTE: Handle gently and quickly to maintain viability. Collagen I coated culture plasticware is recommended.

1. Place vial in a 37˚C water bath, hold and rotate vial gently until the contents are completely thawed.
2. 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.
3. Using a pipette, gently transfer contents of vial to a sterile 15 mL conical tube.
4. Wash vial with 5 mL MCST250 medium and add this wash to conical tube.
5. Centrifuge the tube at 250 x g for 5 minutes.
6. After centrifugation, aspirate medium and re-suspend the contents in 1mL (or less as desired to achieve accurate counts) fresh MCST250 medium.
7. Count the cells using the Trypan Blue Exclusion Assay (See section VIII for recommended counting protocol).
8. Add sufficient MCST250 medium to seed the cells at a density of 4,000 cells/cm^2 on collagen I coated plates for Passage-1 Stellates or 8,000-10,000 cells/cm^2 for Passage-0 Stellates using volumes appropriate for the well or plate size being used.
9. 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.

VII. SubCulturing

1. Subculture cells when they have reached 90% confluency.
2. Warm MCST250 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 to flask and incubate in a 37˚C incubator for 3-5 minutes, or until the cells detach.
4. At the end of trypsinization, wash cells off flask with an appropriate amount of medium.
5. Transfer to centrifuge tube and centrifuge at 250 x 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.

VIII. Cell Counting Procedure

NOTE: To achieve accurate cell counts, it is recommended to use a manual Trypan Blue Exclusion Method. Trypan Blue Exclusion Method must be used to accurately determine viability and yield. Use of any other method may result in viability and yield different from that shown on the lot specific CofA.

1. To a clean microfuge tube, add 50µL of 0.4% Trypan Blue Solution, 350µL of Stellate Growth medium and 100uL of cell suspension. This results in a 1:5 fold dilution of your cells. If a different dilution is desired, volumes may be adjusted as long as the 0.4% Trypan Blue represents no more than 10% of the total volume (e.g. 0.04% final Trypan Blue).
2. Determine cell viability using the formula below.

   Eq. 1: 100 x (Live cell count ÷ Total cell count) = Viability%

3. Determine total viable cell yield using the formula below.

   Eq. 2: Viable cell count ÷ Quadrants counted x Dilution factor x 10000 x Current volume (mL) = Viable cell yield

   Example: 100 cells ÷ 4 quadrants x 5 x 10000 x 3mL total volume = 3,750,000 cells

IX. Product Warranty

Cultures have a finite lifespan *in vitro.*

Lonza guarantees the performance of cells only if appropriate media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

X. Quality Control

For detailed information concerning QC testing, please refer to the Certificate of Analysis.

When placing an order or contacting Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all cell culture products, refer to the Lonza website or our current catalog. To obtain a catalog, additional information or Scientific Support, you may contact Lonza by web, e-mail or telephone. Contact details are listed at the top of this document.

XI. Safety Statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* diagnostic procedures.

WARNING: LONZA PRIMARY CELL PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th ed. If you require further information, please contact your site safety officer or Scientific Support.