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Cryo Human Stellate Cells 1e6 per vial (HUCLS-1M or HUCLS1), Cryo Human Stellate Cells 2e5 per vial (HUCLS-200K), Cryo Human Stellate Cells 1e5 per vial (HUCLS)

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 or references citing the use of these products, please visit Lonza's Knowledge Center:

https://knowledge.lonza.com/

II. Required reagents and materials

(components sold separately)

1. Cryo Human Stellate Cells 1e6 per vial (HUCLS-1M or HUCLS1), Cryo Human Stellate Cells 2e5 per vial (HUCLS-200K), Cryo Human Stellate Cells 1e5 per vial (HUCLS)
2. StCM™ Stellate Cell Culture BulletKit™ (Lonza catalog number MST-500BK)
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. Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells, remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Scientific Support at the email address provided in the header to this page.

3. Lonza's BulletKit™ Medium instructions: upon arrival, store Basal Medium at 2° - 8°C and SingleQuots™ Kit at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 2° - 8°C and added to the basal medium within 72 hours of receipt. After the SingleQuots™ Kit Supplements are added to basal medium use within one month. Do not re-freeze.

IV. Preparation of BulletKit™ Medium

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire contents to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label (avoid covering the basal medium lot # and expiration date) to avoid confusion or possible double supplementation. **After adding supplements, the complete medium has a shelf life of one month. Do not freeze medium.**
5. Record the new expiration date on the label based on the shelf life.

NOTE: If there is concern that the sterility was compromised during the supplementation process, newly prepared medium may be re-filtered with a 0.2-micron filter to assure sterility. Routine filtration/re-filtration is not recommended.

V. Thawing and plating cells

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

1. Warm media in a 37°C water bath prior to thawing cryopreserved cells.
2. Transfer 9 mL of warmed completed StCM™ Stellate Cell Culture BulletKit™ Medium into a sterile 15 mL conical tube.
3. Place vial in a 37˚C water bath, hold and rotate vial gently until the contents are thawed with a sliver of ice remaining.
4. 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.
5. Using a pipette, gently transfer contents of vial to the sterile 15 mL conical tube.
6. Wash vial with 1 - 2 mL medium from 15 mL conical tube and add this wash back to the conical tube.
7. Centrifuge the tube at 300 x g for 5 minutes.
8. After centrifugation, aspirate medium and re-suspend the contents in 0.2 - 1 mL (or less as desired to achieve accurate counts) fresh completed StCM™ Stellate Cell Culture BulletKit™ Medium.
9. Count the cells using the Trypan Blue Exclusion Assay (See section VIII for recommended counting protocol).
10. Add sufficient completed StCM™ Stellate Cell Culture BulletKit™ Medium to seed the cells at a density of 4,000 - 5,000 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.

VI. Maintenance

1. For best results, do not disturb the culture for at least 12 hours after seeding.
2. Change medium the next day to remove any residual DMSO or unattached cells, then every 2 - 3 days thereafter.

VII. Subculturing

1. Subculture cells when they have reached 90% confluence.
2. Warm completed StCM™ Stellate Cell Culture BulletKit™ 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 300 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 4-5,000 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 80µL of 0.4% Trypan Blue Solution,and 20uL of cell suspension. This results in a 1:5 fold dilution of your cells. If a different dilution is desired, volumes may be adjusted.
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. Ordering information

Cryopreserved cells

Cat. no.	Product	Size
HCLS	Cryo Human Stellate Cells 1e5 per vial	≥100,000 viable cells/vial
HCLS-200K	Cryo Human Stellate Cells 2e5 per vial	≥200,000 viable cells/vial
HUCLS1	Cryo Human Stellate Cells 1e6 per vial	≥ 1,000,000 cells/vial
HUCLS-1M	Cryo Human Stellate Cells 1e6 per vial	≥ 1,000,000 cells/vial

HLKC BulletKit™ Culture Medium (must be purchased separately)

Cat. no.	Product	Size
MST-500BK	StCM™ Stellate Cell Culture BulletKit™	Contains 500mL bottle and SingleQuots
MST-500	StBM™ Stellate Cell Culture Basal Medium	500 mL bottle
MST-500SQ	StCM SingleQuots™ Stellate Cell Culture Supplements and Growth Factors	SingleQuots

X. 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.

XI. 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.

XII. 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.