Cryopreserved Primary Human Stellate Cells

FOR NON-CLINICAL RESEARCH USE ONLY

Product Description

LifeNet Health's primary human stellate cells are isolated from donated human tissue, resulting from the generous gift of an individual or their family. The cells are isolated using a refined cell isolation technique resulting in high-quality cells suitable for a wide range of research applications.

Indications for Use

LifeNet Health's primary human stellate cells are for research use only. The cells are not intended for human use, for any *in vitro* diagnostic procedures, or for therapeutic procedures. Transfer or resale of any LifeNet Health cells or products is prohibited without the written consent of LifeNet Health.

Warnings and Precautions

Observe universal precautions when handling human-derived tissues and cells as they are potentially biohazardous. Refer to the guidelines set forth in Occupational Safety and Health Standards for handling blood, tissues, body fluids, or other potentially infectious materials. Follow institutional guidelines for the collection and disposal of all solid and liquid waste that has been in contact with these products.

Donor Screening and Testing

Donor authorization for non-clinical research use of these cells was appropriately obtained and documented by LifeNet Health. All donors are tested and confirmed negative for the following infectious diseases: Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Syphilis, and Toxoplasmosis.

Storage Requirements

The distributor, intermediary and/or end-user is responsible for storing these cells under appropriate conditions prior to further distribution or use. LifeNet Health ships frozen cells on dry ice or in the vapor phase of liquid nitrogen (-135°C to -190°C) depending on the quantity of vials being shipped. On receipt, immediately transfer frozen cells to storage in the vapor phase of liquid nitrogen (-135°C to -190°C) until ready for experimental use.

Final Product Testing

Each LifeNet Health primary human stellate cell lot is fully characterized to determine post-thaw results including cell viability and yield, morphological integrity, and population doubling rate. Each lot is tested for purity using immunocytochemistry (ICC) to determine positive expression for standard cell markers. Each cell culture is tested and determined negative for bacteria, yeast, fungi, and mycoplasma. A Certificate of Analysis (CoA) is available for each lot and includes comprehensive donor history, histological images with pathology results, characterization data, and respective cell culture images.

Complaints and Returns

For further information or returns or to report a complaint, please contact your authorized distributor or LifeNet Health Client Services (available 24 hours a day) at 1-888-847-7831 (inside the U.S.) or 00+1-757-464-4761 ext. 2000 (outside the U.S.) and have the product code and lot number available (see CoA).

Human Stellate Cell Protocols

It is important to read and understand the following instructions prior to use. Improper handling may adversely affect cell quality and performance.

Recommended Supplies and Reagents

Complete Media:	**DMEM + 10% FBS + 1% Antibiotic/Antimycotic** DMEM: Life Technologies #11965-092 (500 mL) Fetal Bovine Serum (FBS): Gemini Bio #100-106 (55 mL) Antibiotic/Antimycotic: Life Technologies #15240-062 (5.5mL)
Wash Buffer:	PBS: Corning/Mediatech #21-040-CM w/o Calcium & Magnesium
Detachment:	Trypsin (0.25%)/EDTA (2.21 mM) in HBSS: Corning/Mediatech #25-053-CL OR TrypLE Express, Gibco/Thermo Fisher cat# 12604-021
Cryopreservation:	Cellbanker® 1 freezing media: Amsbio #11888
Culture Vessels:	Falcon/Corning 100 mm dish #353003
Freezing Container:	Nalgene "Mr. Frosty" #5100-0001

Thawing Procedure

1. Warm Complete Media in 37°C water bath; clean exterior of bottle with 70% ethanol before use
2. In Biological Safety Cabinet (BSC): Aliquot 9 mL of warmed Complete Media into sterile 15 mL centrifuge tube
3. Hold cryovial(s) in a 37°C water bath to thaw without submerging the cap in water (hold until only a sliver of ice remains, approximately 1.5-2 minutes)
4. Remove from water bath and clean exterior of vial(s) with 70% ethanol before placing into BSC
5. In BSC: Transfer entire contents of cryovial(s) into the 15 mL tube, containing 10 mL of warm Complete Media, using a 1.0 mL sterile pipet
6. In BSC: Remove 1 mL of the cell suspension from the 15 mL tube and use it to rinse the cryovial(s) to capture residual cells; return the 1 mL rinse to the 15 mL tube
7. In BSC: Cap the 15 mL tube and mix gently

Centrifuge Procedure

1. Centrifuge cells at 500 x g for 5 minutes at room temperature
2. IN BSC: Gently aspirate supernatant, then re-suspend pellet in an appropriate volume of fresh Complete Media

Plating Procedure

1. Enumerate viable cells using lab standard methods and procedures
2. IN BSC: Pre-fill culture vessels with ½ standard total media volume
3. IN BSC: Dispense cells at a seeding density of 5,000 cells/cm^2 or 280,000 cells per 100 mm plate and immediately swirl gently to distribute
   Note: stellate cells adhere quickly to culture dishes and best results are achieved when cells are distributed by swirling immediately after dispensing
4. IN BSC: Add sufficient volume to reach total culture vessel volume with warmed Complete Media (suggested media volume: 0.16-0.18 mL/cm^2 of vessel surface growth area)
5. Place culture vessels in humidified 37°C incubator @ 5% CO_2

Cell Culture Maintenance Procedure

1. IN BSC: Aspirate and replace culture media every 3-4 days using 10 mL of fresh warmed Complete Media per 100 mm dish or the appropriate volume of fresh warmed Complete Media to each vessel (suggested maintenance media volume: 0.16-0.18 mL/cm^2 of vessel surface growth area)
2. Continue this schedule until cells reach >85% confluence, at which point they should be detached from the culture dish and passaged or cryopreserved

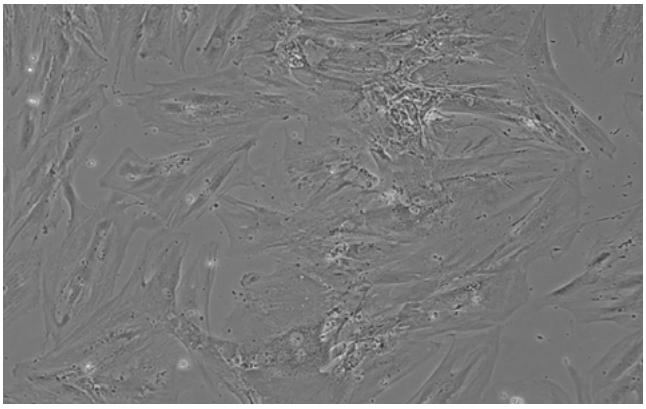

Figure 1. HL150001SC, p4 are shown at harvest (100x)

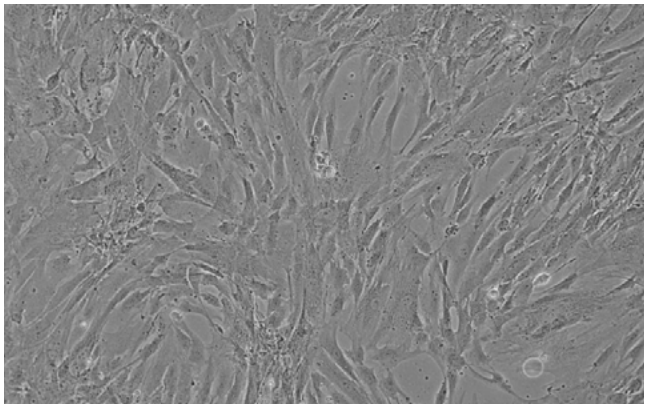

Figure 2. HL150002SC, p4 are shown at harvest (100x)

Cell Detachment and Passage Procedure

1. Warm Complete Media and Trypsin/EDTA in 37°C water bath.
2. IN BSC: Aspirate media from culture vessels and wash each vessel 2x with PBS (Recommended at least 3 mL PBS/100mm culture vessel)
3. IN BSC: Add 0.038 mL/cm^2 Trypsin/EDTA (3 mL per 100mm vessel) and rock gently to ensure uniform coverage
4. Incubate in humidified incubator (37°C, 5% CO_2) for 2-5 minutes with gentle agitation approximately every 2 minutes, checking frequently for detachment; when cells begin to detach remove all culture vessels from the incubator and place in BSC
5. IN BSC: Using a sterile pipet and the trypsin already in the vessels, rinse the culture vessels several time to remove the remaining attached cells; the use of cell scrapers or jarring agitation of culture vessels is not recommended
6. IN BSC: Add 0.091 mL/cm^2 of Complete Media to quench trypsin (5 mL per 100 mm dish)
7. IN BSC: Collect cell suspensions into sterile 50 mL conical tube(s)
8. IN BSC: Add another 0.091 mL/cm^2 of Complete Media to each dish to collect any remaining cells; add it to the 50 mL conical tubes
9. Centrifuge at 500 x g for 5 minutes
10. IN BSC: Aspirate supernatant from tube(s) and resuspend the pellet(s) in 5-10 mL Complete Media and combine into one 50 mL conical tube
11. Obtain cell counts and assess viability using an appropriate method
12. IN BSC: Dispense cells at a seeding density of 4,500 – 5,000 cells/cm^2, or 250,000 cells per 100 mm plate and swirl gently to distribute
13. IN BSC: Add sufficient volume to reach total culture vessel volume with warmed Complete Media
14. Place culture vessels in humidified incubator (37°C, 5% CO_2)

Cryopreservation Procedure

1. Place Cellbanker®1 freezing media and estimated number of cryovials on ice to chill
2. Follow steps 1-8 of the Cell Detachment and Passage procedure
3. Centrifuge harvested cell suspension(s) at 500 x g for 5 minutes
4. IN BSC: Aspirate supernatant from tube(s) and resuspend the pellet(s) in an appropriate volume of room temperature Complete Media
5. Obtain cell counts and assess viability using an appropriate method
6. Centrifuge at 500 x g for 5 minutes
7. IN BSC: Aspirate supernatant and place tube with cell pellet on ice
8. IN BSC: Resuspend cells at a desired freezing concentration, but no less than 1 x 10^6 cells/mL in ice-cold Cellbanker®1 freezing media
9. Aliquot cells into pre-chilled 1 mL cryovials @ 1 mL/vial
10. Transfer vials to a Mr. Frosty freezing container and place in a -80°C freezer overnight and relocate to liquid nitrogen storage at 24 hours

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