NEUROMICS

Human Hepatic Stellate Cells (HHSCs) – NASH Donor

Catalog #: HHSC001

Cell #: >1.0x10^6 cells

Storage: Liquid Nitrogen until ready for culture.
While Culturing keep in 37°C CO_2 incubator

Product Format: Frozen Vial

GENERAL INFORMATION

Human Hepatic Stellate Cells (HHSCs) from NASH donors are isolated from human liver tissue with a confirmed histological diagnosis of Non-Alcoholic Steatohepatitis (NASH). These cells are cryopreserved at the earliest passage to preserve their critical in vivo phenotype.

Hepatic Stellate Cells are the principal effector cells in liver fibrosis. In the healthy liver, they reside in a quiescent state, storing Vitamin A. Upon liver injury, as in NASH, they undergo "activation," transforming into proliferative, contractile, and fibrogenic myofibroblasts. HHSCs from a NASH background are pre-activated and provide an unparalleled in vitro model for studying the mechanisms of progressive fibrogenesis, screening anti-fibrotic therapeutics, and investigating the pathogenesis of NASH.

*Differentiation efficiency may vary due to the inherent biological variability of the obese donor phenotype.

These cells exhibit biological characteristics associated with the obese donor phenotype, which may impact their growth, differentiation, and metabolic behavior compared to cells from lean donors. It is the user's responsibility to determine the suitability of this product for their specific application.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

KEY FEATURES & BENEFITS

- **Disease-Relevant Phenotype:** Isolated from a verified NASH liver, providing cells that are primed for fibrogenesis and reflect the in vivo disease state.
- **Key Drivers of Fibrosis:** Central model for studying collagen deposition, alpha-Smooth Muscle Actin (α-SMA) expression, and pro-fibrotic signaling pathways.
- **Characterized Activation State:** Verified for high expression of activation markers (α-SMA, Collagen I) and reduced Vitamin A lipid droplets compared to healthy HHSCs.
- **Functionally Validated:** Each lot is tested for viability, proliferation, and expression of key fibrotic markers.

APPLICATIONS

- **NASH & Liver Fibrosis Research:** Model the key cellular processes driving disease progression.
- **Anti-Fibrotic Drug Discovery:** Screen and validate novel compounds aimed at inhibiting HSC activation, proliferation, or collagen production.
- **Mechanistic Studies:** Investigate TGF-β, PDGF, and other pro-fibrotic signaling pathways.
- **Cell-Cell Interactions:** Establish co-culture models with hepatocytes, Kupffer cells, or liver sinusoidal endothelial cells to study cross-talk in NASH.
- **Extracellular Matrix (ECM) Biology:** Study the composition and remodeling of the fibrotic niche.

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QUALITY CONTROL SPECIFICATIONS

Each lot is tested to meet the following release criteria:

Parameter	Specification	Test Method
Viability (Post-Thaw)	≥ 75%	Trypan Blue Exclusion
Cell Yield per Vial	≥ 1 Million Cells	Cell Count
Morphology	Elongated, myofibroblast-like (activated)	Microscopic Evaluation
Immunofluorescence	Positive: Alpha-Smooth Muscle Actin (α-SMA), Vimentin, Collagen I	IF Microscopy
Proliferation Assay	Positive for population doubling in response to pro-fibrotic stimuli (e.g., TGF-β1)	Functional Assay
Microbiological Sterility	No Growth	USP <71>
Mycoplasma	Negative	PCR Method

TYPICAL DONOR DEMOGRAPHICS & CLINICAL HISTORY

Donors are carefully selected and characterized. A typical profile includes:

- **Diagnosis:** Histologically confirmed NASH (NAFLD Activity Score typically ≥5 with fibrosis stage 1-3).
- **Age Range:** 35 - 65 years
- **Sex:** Male and Female donors available
- **Common Comorbidities:** Obesity, Type 2 Diabetes, Dyslipidemia, Hypertension.
- **Exclusion:** Significant alcohol consumption, other liver diseases (HBV, HCV, AIH).

HANDLING OF ARRIVING CELLS

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells into a -80°C freezer for short-term storage or liquid nitrogen tank for long-term storage.

3. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

4. When handling cell cultures, the application of stringent aseptic techniques is imperative. Maintaining a contamination-free environment is crucial for preserving the integrity and reliability of experimental results. This involves not only cleanliness but also the use of sterile tools, media, and working environments to prevent contamination from airborne particles, microbial agents, and other sources.

MEDIUM

We recommend Hepatic Stellate Cell Growth Medium (cat. HHSCM001) for these cells. The growth medium contains ingredients to support cell growth.

PROTOCOL FOR THAWING THE CELLS

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

1. Quick Thaw: Remove the vial from liquid nitrogen and immediately place in a 37°C water bath. Gently agitate until only a small ice crystal remains (≈1-2 minutes).

2. Decontaminate: Spray the vial with 70% ethanol before transferring to a biosafety cabinet.

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3. Transfer and Dilute: Gently transfer the cell suspension to a 15 mL conical tube. Slowly add 5-9 mL of pre-warmed complete growth medium drop-wise to dilute the cryoprotectant (DMSO).

4. Centrifuge: Spin at 200 x g for 5 minutes.

5. Resuspend and Plate: Aspirate the supernatant and gently resuspend the cell pellet in fresh, pre-warmed complete growth medium. Plate cells into a culture flask pre-hydrated with 2-3 mL of medium.

    a. Recommended Seeding Density: 10,000 - 15,000 cells/cm².

6. Incubate: Place the culture vessel in a humidified 37°C incubator with 5% CO_2.

7. Medium Change: Replace the medium after 24 hours to remove any non-adherent cells or debris, and then every 2-3 days thereafter.

SUBCULTURING PROCEDURE

1. Wash: Once cells are 80-90% confluent, aspirate the medium and rinse the cell layer gently with PBS.

2. Trypsinize: Add enough pre-warmed Trypsin/EDTA solution to cover the cell layer (e.g., 2 mL for a T-75 flask). Incubate at 37°C for 3-5 minutes. Observe under a microscope until cells round up and detach.

3. Neutralize: Add an equal volume of pre-warmed complete growth medium to neutralize the trypsin.

4. Centrifuge and Resuspend: Transfer the cell suspension to a tube and centrifuge at 200 x g for 5 minutes. Aspirate the supernatant and resuspend in fresh medium.

5. Re-plate: Seed cells into new culture vessels at a recommended split ratio of 1:2 to 1:3.

Note: These cells are activated and may proliferate faster than quiescent HHSCs. Avoid letting cultures become over-confluent, as this can lead to spontaneous differentiation and reduced responsiveness.

CRYOPRESERVATION

To bank cells, subculture as described and resuspend the pellet at 1-1.5 x 10^6 cells/mL in a cryopreservation medium (e.g., FBS with 10% DMSO). Use a controlled-rate freezer or an isopropanol freezing container at -80°C for 24 hours before transferring to liquid nitrogen.

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