User Guide

Normal Human Hepatic Stellate Cells

HLP301-100K, HLP301-200K, HLP301-250K, HLP302-1M

Store in Liquid Nitrogen.

FOR RESEARCH USE ONLY

Not for Use in Diagnostic Procedures. Not for Human or Animal Consumption.

Product Overview

Primary Human Stellate cells are isolated from whole human livers that are deemed not suitable for liver transplantation and have received consent to be donated for research. Hepatic Stellate Cells (HSCs; also called as vitamin A-storing cells, lipocytes, interstitial cells, fat-storing cells, Ito cells) exist in the space between Parenchymal cells and Sinusoidal Endothelial cells of the hepatic lobule. Stellate cells store 80% of vitamin A in the whole body as retinyl palmitate in lipid droplets in the cytoplasm and are responsible for Vitamin A homeostasis. Stellate cells have a star-like appearance and upon activation can acquire contractile myofibroblast-like morphology which is an essential step in fibrosis. In fibrosis, activated stellate cells display a loss of stored vitamin A, and increased collagen synthesis along with other extracellular matrix components. Each lot is guaranteed for post-thaw cell viability of ≥ 70%. All the lot-specific information including donor information can be obtained via Certificate of Analysis (CoA) upon request.

Quality Control Testing

- Post-thaw viability ≥ 70%, with a yield of ≥ 100K, 200K, 250K, or 1M viable cells per vial.
- Cell marker analysis: αSMA, Vimentin and Vinculin.
- Each donor is tested negative for: HIV, Hepatitis B, Hepatitis C, and syphilis*.
- The culture is tested negative for: Gram +, Gram −, Mycoplasma and Fungi.

*No known test can offer complete assurance that the viruses that cause HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B and hepatitis C are not present. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-2 (Biosafety Level 2) or higher.

Materials Provided

Normal Human Hepatic Stellate Cells:

One (1) vial containing ≥ 100K, 200K, 250K or 1M cells.

Materials Required (Not Provided)

- Collagen Type I, Rat Tail (Cat No. #08-115).
- Tissue culture treated Multiwell plates.
- Trypsin solution, 0.25% with EDTA (Cat No. #T4049).

Please see Protocols for media components.

Storage

Upon receipt, immediately store cryovial(s) in vapor phase liquid nitrogen.

Protocols

All protocols are performed within a Class II laminar flow biohood and with an aspirator unless otherwise specified. Incubators are humidified and are set to 37 °C and 5% CO_2.

PPE should be worn such as gloves, lab coat, and safety glasses.

Preparing Collagen Coated Plate

1. Dilute the collagen to a final concentration of 56 g/mL in sterile 70% ethanol and gently mix until the collagen is solubilized.
2. Add the appropriate volume of the collagen/ethanol mixture to each well to completely cover the bottom of wells.
3. Gently move the cell culture plate until the collagen/ethanol mixture evenly coats the inside of the well.
4. Air dry plates in a laminar flow hood. Leave cell culture plate over night with the cover ajar to allow airflow and prevent condensation.

Preparing Stellate Medium for Human Hepatic Stellate Cells

Formulations for human hepatic stellate cell media are readily available from literature. Below is an example media from one of the publications*. All components listed below are available at SigmaAldrich.com.

Components	Cat No.	Working Stock	Final Dilution	Final Concentration	Final Volume (mL)
DMEM. High Glucose	D5796-500ML	-	-	1x	445
FBS	ES-009-B	-	-	10%	50
Antibiotic-antimycotic 100x	A5955	100x	100x	1x	5
				Total volume	500

* Liu, X., Brenner, D.A., Kisseleva, T. (2023). Human Hepatic Stellate Cells: Isolation and Characterization. In: Weiskirchen, R., Friedman, S.L. (eds) Hepatic Stellate Cells. Methods in Molecular Biology, vol 2669. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-3207-9_13

Thawing and Plating Stellate Cells

Handle gently and quickly to maintain viability.

Collagen I coated culture ware is required for culturing stellate cells (see above, Preparing Collagen Coated Plate).

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 warm 5 mL stellate medium and add this wash to conical tube.
4. Centrifuge the tube at 250 x *g* for 5 minutes. After centrifugation, aspirate medium and re-suspend the contents in fresh stellate medium.
5. Perform cell count.
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 growth medium the next day to remove any residual DMSO or unattached cells, then every other day thereafter.

Instruction 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 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.

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