User Guide

Normal Human Hepatic Sinusoidal Endothelial Cells

HLP601-500K **HLP602-500K** **HLP602-1M**

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption

Product Overview

Human Hepatic Sinusoidal Endothelial Cells (HHSECs) are isolated from human liver obtained via the gift of organ donation from donor tissue that is not suitable for organ transplantation. Each donor has confirmed documentation on file allowing for research use of any non-transplantable organs or tissues. The cells are isolated through collagenase digestion and selective cell culture media. These cells are a valuable tool for the study of liver physiology and pathophysiology. They are specialized endothelial cells that participate in receptor-mediated clearance of endotoxin, bacteria, and other compounds. They also regulate inflammation, leukocyte recruitment, and host immune responses to pathogens. Frozen HHSECs are cryopreserved at the end of the primary culture. 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 of ≥ 70%, with a yield of ≥ 500K or 1M viable cells per vial.
- Cell marker analysis: CD146, vWF, CD105 and CD31.
- 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 Sinusoidal Endothelial Cells:

One (1) vial containing 500K or 1M cells per vial.

Materials Required (Not Provided)

- Collagen Type I, Rat Tail (Cat No. 08-115).
- Tissue culture treated Multiwell plates.
- Dulbecco's PBS, without Calcium and Magnesium (DPBS) (Cat No. D8537-500ML).
- 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 56µg/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 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 Medium for Human Sinusoidal Endothelial Cells

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

Components	Cat No.	Working Stock	Final Dilution	Final Concentration	Final Volume (mL)
Human Endothelial Medium	11111044**			1x	450
AB Human Serum	H3667-100ML			10%	50
Human VEGF	SRP3182-10UG	0.1 mg/mL	10,000x	10 ng/mL	0.05
Human HGF	H5791-10UG	0.1 mg/mL	10,000x	10 ng/mL	0.05
				Total volume	500

* Vascular Adhesion Protein-1 Mediates Adhesion and Transmigration of Lymphocytes on Human Hepatic Endothelial Cells. Patricia F. Lalor; Sarah Edwards; Gillian McNab; Marko Salmi; Sirpa Jalkanen; David H. Adams J Immunol (2002) 169 (2): 983–992.

** Available from Gibco.

Thawing and Plating Human Hepatic Sinusoidal Endothelial Cells

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 sterile field. Remove cap, being careful not to touch the interior threads with fingers.
2. Using a pipette, gently transfer contents of vial to a 15 mL conical tube. Wash vial with 5 mL medium and add wash to conical tube.
3. Centrifuge tube at 300 x *g* for 5 minutes. After centrifugation, aspirate medium and re -suspend the contents in medium.
4. Count the cells.
5. For expansion, seed the cells at a density of 5,000 cells/cm^2 on Collagen I coated plates.
6. 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.
7. Continue to change media every other day until ready for use.

Instructions for Sub-Culturing Hepatic Sinusoidal Endothelial Cells

1. Subculture cells when they have reached 70-80% confluency.
2. Warm medium, 0.25% trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium and Magnesium (DPBS) to room temperature.
3. Aspirate medium, then rinse cells with DPBS. Add Trypsin solution into the flask and incubate in a 37 °C incubator for 3-5 minutes, or until the cells detach.
4. At the end of typsinization, collect cells from flask with an appropriate amount of medium.
5. Transfer the cells to centrifuge tube, centrifuge at 300 x *g*, room temperature for 5 minutes.
6. After centrifugation aspirate medium, re-suspend and count cells for seeding.
7. Seed the cells at a density of 5,000 cells/cm^2 on Collagen I coated plates.

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