User Guide

Normal Human Intrahepatic Biliary Epithelial Cells

Cholangiocytes

HLP501-250K, HLP501-500K, HLP501-1M

Store in Liquid Nitrogen.

FOR RESEARCH USE ONLY

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

Product Overview

Primary Human Cholangiocytes, also known as Human Intrahepatic Biliary Epithelial Cells, are the epithelial cells that line the intrahepatic bile ducts. These cells are important in modification of the ductal bile and are targeted in multiple liver diseases, such as primary biliary cirrhosis, cholangiocarcinoma and sclerosing cholangitis. Primary Human Intrahepatic Biliary Epithelial Cells 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. These cells 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 ≥250 K, 500 K, or 1 M viable cells per vial.
- Cell surface marker analysis: EpCam (CD326) and cytokeratins (8, 18, and 19).
- 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 Intrahepatic Biliary Epithelial Cells (Cholangiocytes):
One (1) vial containing 250 K, 500 K, or 1 M cells per vial.

Materials Required (Not provided)

Catalog numbers in ( ) can be ordered from SigmaAldrich.com unless otherwise noted.

- Collagen Type I, Rat Tail (08-115)
- Tissue culture treated multi-well plates
- Dulbecco's PBS, without Calcium & Magnesium (DPBS) (D8537-500ML)
- Trypsin solution, 0.25% with EDTA (T4049)
- Please see Protocol for media components

Storage and Stability

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 µg/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 Cholangiocytes.

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

Components	Catalog Number	Working Stock	Final Dilution	Final Concentration	Final Volume (mL)
DMEM/F-12 mix	D6421-500ML	-	-	1X	439
FBS	ES-009-B	-	-	10%	50
Hydrocortisone	H0888-1G	1 mg/mL**	500X	2 µg/mL	1
Insulin	I9278-5ML	10 mg/mL	2,326X	4.3 µg/mL	0.215
Cholera Toxin	C8052-0.5MG	0.1 mg/mL	10,000X	10 ng/mL	0.05
Triiodo-L-thyronine	T6397-100MG	20 µg/mL (30 µM)***	15,000X	2 nM	0.033
Human EGF	E5036-200UG	0.1 mg/mL	10,000X	10 ng/mL	0.05
L-glutamine	TMS-002-C	200 mM	100X	2 mM, 1X	5
Pen/Strep	P7539	100X	100X	1X	5
				Total volume	**500**

* CXC Chemokine Ligand 16 Promotes Integrin-Mediated Adhesion of Liver-Infiltrating Lymphocytes to Cholangiocytes and Hepatocytes within the Inflamed Human Liver. Mathis Heydtmann; Patricia F. Lalor; J. Albertus Eksteen; Stefan G. Hübscher; Mike Briskin; David H. Adams J Immunol (2005) 174 (2): 1055–1062.

** Dissolve in 100% EtOH.

*** To prepare 20 µg/mL stock solution: add 1mL 1N NaOH per mg 3,3', 5-triiodo-L-thyronine; gently swirl to dissolve. To this, add 49 mL sterile medium per mL 1N NaOH added.

Thawing and Plating Human Cholangiocytes

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 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 containing 5 mL of Cholangiocyte medium. Wash vial with 1 mL of the medium and add the wash to the same conical tube.
3. Centrifuge tube at 300 x *g* for 5 minutes. After centrifugation, aspirate medium and re-suspend the contents in medium. Perform a cell count.
4. For expansion, seed the cells at a density of 5,000 cells/cm^2 on collagen I coated plates.
5. For best results, do not disturb the culture for at least 12 hours after seeding. Change the medium the next day to remove any residual DMSO or unattached cells.
6. Feed cells fresh Cholangiocyte medium every other day until ready for assay or expansion.

Sub-Culturing Cholangiocytes

1. Subculture cells when they have reached 70-80% confluency.
2. Warm Cholangiocytes medium in a 37 °C water bath.
3. Make sure 0.25% trypsin solution, and Dulbecco's Phosphate Buffered Saline, without Calcium & Magnesium (DPBS) are at room temperature.
4. Aspirate the medium and 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.
5. As soon as the cells detach, wash cells from flask using two (2) times the volume with Cholangiocyte medium. Transfer to centrifuge tube, centrifuge at 250 x *g* for 5 minutes. After centrifugation aspirate medium, re-suspend and count cells for seeding.
6. Seed the cells at a density of 5,000 cells/cm^2 in collagen I coated plates.

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