User Guide

Normal Human Characterized Suspension Hepatocytes

HLP101-3M HLP101-4M HLP101-8M

Store in liquid nitrogen

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption

Product Overview

Primary Human Suspension Hepatocytes are isolated from whole human livers that are deemed not suitable for liver transplantation and have received consent to be donated for research. The cell composition consists of a homogenous population of hepatocytes. Each lot is guaranteed for a post-thaw viability of ≥70%. Human hepatocytes are ideal for the studies of enzyme induction, toxicity, drug screening, transporter efflux activity, and potential drug-drug interactions. 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 ≥3, 4, or 8 million viable cells per vial.
- Profiling Data of Phase I (CYP) and Phase II (UGT, SULT) Enzymes.
- 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 Characterized Suspension Hepatocytes:

One (1) vial containing ≥ 3, 4, 8 million cells. Store in liquid nitrogen.

Materials Required (Not provided)

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

- Tissue culture treated multi-well plates or tubes
- Please see Protocol 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 1X Hepatocyte Plating Medium (HPM) for Human Hepatocytes

All components listed below are available at SigmaAldrich.com.

Hepatocyte Plating Media (HPM)

Components	Catalog Number	Working Stock	Final Dilution	Final Conc.	Final Volume (mL)
DMEM. High Glucose	D1145-500ML			1 x	464
FBS	ES-009-B			5%	25
Dexamethasone	D4902-25MG	2mM	2,000x	1 µM	0.25
Insulin	I9278-5ML	10mg/mL	2,500x	4 µg/mL	0.2
Gentamicin	G1272	10mg/mL	1,000x	10 µg/mL	0.5
L-glutamine	TMS-002-C	200mM	100x	2 mM, 1x	5
NEAA	TMS-001-C	10mM	100x	0.1 mM, 1x	5
				Total Volume	500

Preparing 1X Maintenance Medium for Human Hepatocytes

All components listed below are available at SigmaAldrich.com.

Maintenance Media

Components	Catalog Number	Working Stock	Final Dilution	Final Conc.	Final Volume (mL)
Williams E	W1878-500ML			1 x	466.4
Human Insulin	I9278-5ML	10 mg/mL	1,600 x	6.25 µg/mL	0.313
Human Transferrin	T0665-50MG	1 mg/mL	160 x	6.25 αµg/mL	3.125
Selenium	S9133-1MG	0.1 mg/mL	16,000 x	6.25 ng/mL	0.031
Dexamethasone	D4902-25MG	2 mM	20,000 x	0.1 µM	0.025
HEPES	H0887-20ML	1 M	66.6 x	15 mM	7.5
BSA, Fatty acid free	A8806-1G	50 mg/mL	40 x	1.25 mg/mL	12.5
linoleic acid	L1012-100MG	50 mg/mL	9,346 x	5.35 µg/mL	0.053
L-Glutamine	TMS-002-C	200 mM	50 x	4 mM	10
Gentamicin	G1272	10 mg/mL	5,000 x	2 µg/mL	0.100
				Total Volume	500

1. For Dexamethasone, dissolve 25 mg into 32 mL of ethanol (100%) to make 2 mM stock.
2. For Linoleic acid, dissolve 100 mg into 2 mL of ethanol (100%) to make 50 mg/mL stock.
3. Once prepared both plating and maintenance media are stable for at least 4 weeks at 4 °C.

Thawing and plating suspension Hepatocytes

4. Fill a 50 mL centrifuge tube with 45 mL of 4 °C Hepatocyte Plating Medium (HPM). Place this into the Bio Safety Cabinet (BSC).
5. Remove hepatocyte vial(s) from storage tank or freezer.
6. Immerse the vial up to the cap into the 37 °C water bath. Be careful not to completely submerge the cap.
7. Gently shake the vial. Shake until the ice pellet has melted to the point of a small spindle, do not fully thaw the cell suspension. It will take approximately 90–120 seconds.
8. Remove the vial from the water bath. Spray with 70% IPA or wipe down with a 70% IPA wipe and transfer into the BSC.
9. Pour the contents of the vial into the 50 mL conical containing HPM.
10. Remove 1mL of this medium and hepatocyte suspension using a pipette and place into the vial, ensuring collection of any remaining cells.
11. Close the 50 mL tube, ensure the cap is tightened, and invert the conical gently 3 to 4 times to ensure resuspension of the hepatocytes.

12. Centrifuge the conical at 100 x *g* for 10 minutes.
13. Remove the conical from the centrifuge and place it into the BSC.
14. Aspirate or pour off the supernatant.
15. Resuspend the cell pellet in 3-4 mL of 37 °C Hepatocyte Maintenance media. Gently resuspend the pellet by slowly rocking the 50 mL conical back and forth, allowing the media to repeatedly wash over the pellet until fully resuspended.
16. Calculate the total cell count and cell viability using the Trypan Blue Exclusion method.
    a. Prepare a 1:10 dilution by combining 400 µL of media with 50 µL of Trypan Blue, then add 50 µL of well-mixed cell suspension.
    b. 1:5 = 350 µL media + 50 µL trypan + 100 µL cell suspension.
    c. 1:2 = 200 µL media + 50 µL trypan + 250uL cell suspension.
        i. Cell viability percentage is calculated using the following formula: LIVE CELLS/(LIVE+DEAD CELLS) X 100.
        ii. Cell yield per mL is calculated using the following formula: (TOTAL LIVE CELLS)/(# SQUARES COUNTED) X 10,000 X DILUTION FACTOR.
17. Add additional Hepatocyte Maintenance Medium to reach the desired cell concentration for experimental design.
18. Proceed with experiment.

Table 1. Example results of Phase I (CYP) and II (UGT, SULT) metabolism activity via ECOD assay, which measures the rate of clearance of 7-ethoxycoumarin and the rate of formation of metabolites 7-hydroxycoumarin sulfate (7-HCS) and 7-hydroxycoumarin glucuronide (7-HCG).

Enzyme	pmole/min/ million cells
CYP1A2	131.50
CYP2A6	10.48
CYP2B6	114.50
CYP2C8	48.00
CYP2C9	111.33
CYP2C19	8.03
CYP2D6	38.33
CYP2E1	106.00
CYP3A4 (Midazolam)	73.33
CYP3A4 (Testosterone)	152.00
GEN	12.13
SULT	4.18
UGT	543.33
AO	64.33

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HLP101ug Rev 01/24