User Guide

Normal Human Characterized Plateable Hepatocytes Spheroid Qualified

HLP104-4M

Store in liquid nitrogen.

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

Product Overview

Primary Human 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 to contain a minimum of 4 million viable cells per vial, with a post-thaw viability of ≥70%, and is tested for 3D spheroid formation at ≥5 days. Human Hepatocytes are ideal for the studies of enzyme induction, toxicity, drug screening, transporter efflux activity, potential drug-drug interactions, and disease modeling. All the lot-specific information including donor information can be obtained via a Certificate of Analysis (CoA) upon request.

Quality Control Testing

- Post-thaw viability of ≥70%, with a yield of ≥4 million viable cells per vial
- Cells are tested for 3D Spheroid formation at ≥5 days
- Induction data of CYP1A2, CYP2B6 and CYP3A4
- 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

  Note: 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.
- The culture is tested negative for: Gram +, Gram -, Mycoplasma and Fungi

Materials Provided

Normal Human Characterized Plateable Hepatocytes Spheroid Qualified

One (1) vial containing ≥4 million cells.

Materials Required (Not provided)

- Ultra Low Attachment Plate (CLS7007)
- 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.

All components listed below are available at SigmaAldrich.com.

Preparing 1X Hepatocyte Plating Medium (HPM) for Human Hepatocytes

Components	Catalogue No.	Working Stock	Final Dilution	Final Concentration	Final Volume (mL)
DMEM. High Glucose	D1145-500ML	-	-	1X	464
FBS	ES-009-B	-	-	5%	25
Dexamethasone	D4902-25MG	2 mM	2,000X	1 µM	0.25
Insulin	I9278-5ML	10 mg/mL	2,500X	4 µg/mL	0.2
Gentamicin	G1272	10 mg/mL	1,000X	10 µg/mL	0.5
L-Glutamine	TMS-002-C	200 mM	100X	2 mM, 1X	5
NEAA	TMS-001-C	10 mM	100X	0.1 mM, 1X	5
				Total Volume	**500**

Preparing Spheroid Medium (SM) for Spheroid Qualified Human Hepatocytes

Components	Catalogue No.	Working Stock	Final Dilution	Final Concentration	Final Volume (mL)
Williams E	W1878-500ML	-	-	1X	416.4
FBS	ES-009-B	-	-	10%	50
Human Insulin	I9278-5ML	10 mg/mL	1,600X	6.25 µg/mL	0.313
Human Transferrin	T0665-50MG	1 mg/mL	160X	6.25 µg/mL	3.125
Selenium	S9133-1MG	0.1 mg/mL	16,000X	6.25 ng/mL	0.031
Dexamethasone	D4902-25MG	2 mM	20,000X	0.1 µM	0.025
HEPES	H0887-20ML	1 M	66.6X	15 mM	7.5
BSA, Fatty acid free	A8806-1G	50 mg/mL	40X	1.25 mg/mL	12.5
Linoleic Acid	L1012-100MG	50 mg/mL	9,346X	5.35 µg/mL	0.053
L-Glutamine	TMS-002-C	200 mM	50X	4 mM	10
Gentamicin	G1272	10 mg/mL	5,000X	2 µg/mL	0.100
				Total Volume	**500**

- For Dexamethasone, dissolve 25 mg into 32 mL of ethanol (100%) to make 2 mM stock.
- For Linoleic Acid, dissolve 100 mg into 2 mL of ethanol (100%) to make 50 mg/mL stock.
- Once prepared media is stable for at least 4 weeks at 4 °C.

Thawing and Preparation of Human Hepatocytes without Centrifugation

1. Pre-warm a bottle of Spheroid Medium (SM) to 37 °C in water bath prior to thawing the cells.
2. Transfer 5 mL of pre-warmed SM into a sterile conical (15-50 mL), leave the conical uncapped.
3. Remove the cryovial and quickly submerge the vial in the 37 °C water bath.
   Note: Do not submerge the cap, only the cell contents portion of the vial.
4. Allow the vial to thaw for 1.5–2 minutes or until a small spindle of ice is present in the cell suspension.
   Note: Do not fully thaw the cell suspension.
5. Remove the vial from the water bath and wipe thoroughly with an alcohol wipe.
6. Remove the cap and transfer the cell suspension into the conical. Rinse the cryovial 2–3 times with warm SM from the conical to capture any remaining cells.
7. Perform a Trypan Blue count using a 1:5 dilution:
   (50 µL of Trypan Blue + 350 µL of SM + 100 µL of cell suspension)
8. Seed the wells with 1,000–5,000 cells per well, 100 µL per well.
   Note: This is for using 96-well plate format.
9. Centrifuge the plate at 100 x *g* for 2 minutes.
10. Gently transfer the plate to the incubator (37 °C, 5% CO_2).
11. Allow 4–7 days for the spheroids to form. Once the spheroids have formed (typically around day 4–6), perform a half-media change by carefully removing 50 µL and then add 50 µL of fresh warm SM per well.
12. Perform half-media changes every 2–3 days thereafter.

Thawing and Preparation of Human Hepatocytes with Centrifugation

For customers preferring to pellet their hepatocytes, HPM either with or without density gradient can be used. When using Percoll® as density gradient, prepare HPM with 27% of 90% Percoll® (i.e., 9 parts Percoll® and 1 part in 10X D-PBS).

1. Fill a 50 mL centrifuge tube with 45 mL of 37 °C Hepatocyte Plating Media (HPM).
2. Remove the cryovial and quickly submerge the vial in the 37 °C water bath.
   Note: Do not submerge the cap, only the cell contents portion of the vial.
3. Allow the vial to thaw for 1.5–2 minutes or until a small spindle of ice is present in the cell suspension.
   Note: Do not fully thaw the cell suspension.
4. Remove the vial from the water bath and wipe thoroughly with an alcohol wipe.
5. Pour the contents of the vial into the 50 mL conical containing HPM.
6. Remove 1 mL of this medium and hepatocyte suspension using a pipette and place into the vial, ensuring collection of any remaining cells.
7. 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.
8. Centrifuge the conical at room temperature with the following rates depending on the plating media:
   - HPM without density gradient: 100 x *g* for 5 minutes
   - HPM with density gradient: 100 x *g* for 10 minutes
9. Remove the conical from the centrifuge.
10. Aspirate or pour off the supernatant.
11. Resuspend the cell pellet in 3~4 mL of 37 °C Spheroid Medium (SM)
12. Proceed to the step 7 of the previous protocol: Thawing and preparation of human hepatocytes without centrifugation.

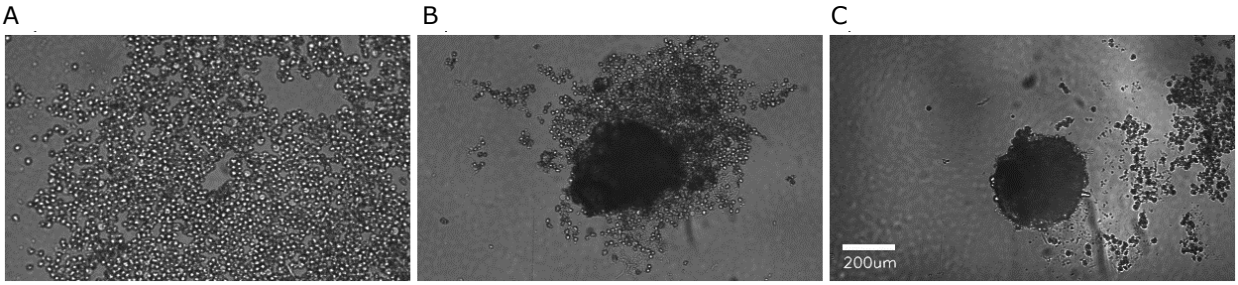

Figure 1. Image of hepatocytes cultured for various days on ULA surface. Cells were seeded at 4,000 cells per well into 96-well ULA plate. Images were taken, **A.** right after seeding and centrifugation, **B.** after 3 days of culturing, and **C.** after 8 days of culturing. All images were taken using Millicell® Digital Cell Imager at 10X magnification.

Notice

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