User Guide

Normal Human Characterized Plateable Hepatocytes

HLP102-3M HLP102-5M HLP103-4M (96 well)

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 for post-thaw cell viability of ≥70%. 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 Certificate of Analysis (CoA) upon request.

Quality Control Testing

- Post-thaw viability of ≥70%, with a yield of ≥3, 4, or 5 million viable cells per vial
- Cells are tested for morphology and growth, with ≥70% confluency for a minimum of 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*
- 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 Plateable Hepatocytes:

One (1) vial containing 3, 4 or 5 million cells.

Materials Required (Not provided)

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

- Collagen Type I, Rat Tail (08-115)
- Tissue culture treated multi-well plates
- 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 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 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	2 mM	2,000 x	1 µM	0.25
Insulin	I9278-5ML	10 mg/mL	2,500 x	4 µg/mL	0.2
Gentamicin	G1272	10 mg/mL	1,000 x	10 µg/mL	0.5
L-glutamine	TMS-002-C	200 mM	100 x	2 mM, 1x	5
NEAA	TMS-001-C	10 mM	100 x	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 ug/mL	0.313
Human Transferrin	T0665-50MG	1 mg/mL	160 x	6.25 ug/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 Hepatocytes without centrifugation

1. Pre-warm a bottle of Hepatocyte Plating Media (HPM) in a 37 °C water bath for at least 15 minutes prior to thawing the cells.
2. Transfer 5 mL of pre-warmed HPM 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 cells 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 dump the cell suspension into the conical. Rinse the cryovial 2-3 times with warm HPM 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 plating media + 100 µL of cell suspension)
8. Adjust the density of the cell suspension as needed using additional Hepatocyte Plating media.
9. Dilute the hepatocyte suspension to the recommended seeding density from the chart below using the HPM.

Plate Format	Seeding Density ($10e^6$) per mL
6-well	0.9-1.1
12-well	0.8-1.1
24-well	0.8-1.1
48-well	0.6-0.7
96-well	0.9-1.0

10. Add the diluted cells to collagen-coated tissue culture plates.
    - 6-well plate, add 2 mL per well.
    - 12-well plate, add 1.0 mL per well.
    - 24-well plate, add 0.5 mL per well.
    - 48-well plate, add 0.25 mL per well.
    - 96-well plate, add 50 µL of Plating media per well, then 50 µL cell suspension per well.
11. Place the plate onto a flat surface and gently shake in a North-South/East-West fashion to evenly disperse the cells. **DO NOT** use a circular motion. Observe the well(s) to ensure that the seeding density is appropriate.
12. Gently place the plates into a 37 °C, 5% CO_2 incubator.
13. Gently shake the plate(s) every 20 minutes after seeding for 1-2 hours. 6-8 hours after the initial seeding, observe the plate under a microscope (10X) to check for cell attachment and flattening. The cells should flatten out enough and begin to form a monolayer with cuboidal shaped cells. If the cells have not flattened out sufficiently, then allow the plate to sit in the incubator overnight.
14. Once the cells have flattened out, replace the media with 37 °C Hepatocyte Maintenance Medium. If using an overlay (Matrigel for example), replace with 4 °C Hepatocyte Maintenance Medium + overlay mixture.
    - For a 6-well plate, add 1.5 mL per well.
    - For a 12-well plate, add 0.8 mL per well.
    - For a 24-well plate, add 0.3 mL per well.
    - For a 48-well plate, add 0.2 mL per well.
    - For a 96-well plate, add 70 µL per well.

      Note: Do not shake 96-well plates.

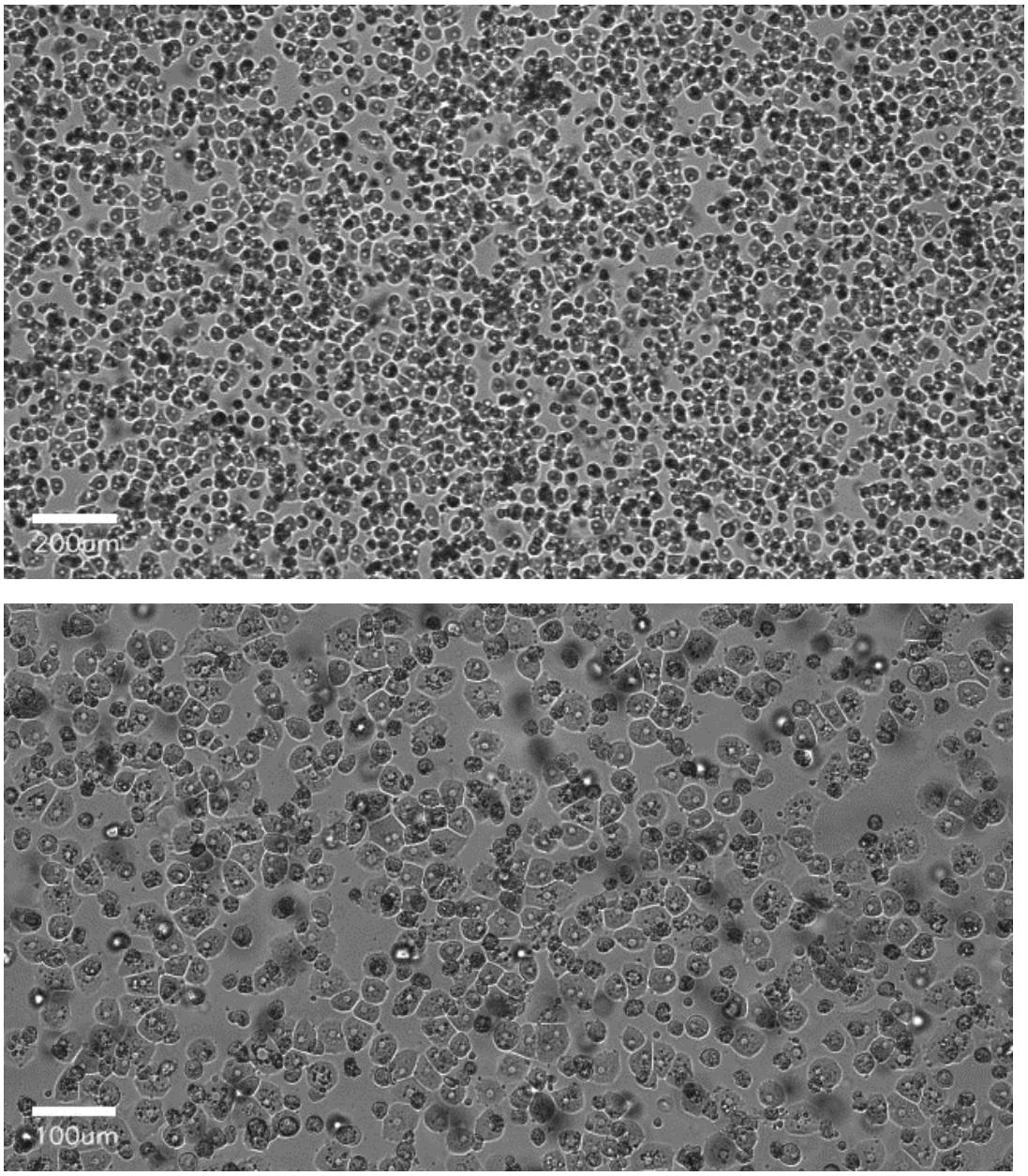
15. Gently place the plates back into the incubator.
16. Replace the Maintenance Medium every 24 hours.

Thawing and plating Hepatocytes with centrifugation*

1. Pre-warm a bottle of Hepatocyte Plating Media (HPM) in a 37 °C water bath for at least 15 minutes.
2. Fill a 50mL centrifuge tube with 45mL of 37°C Hepatocyte Plating Media (HPM).
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. Pour the contents of the vial into the 50mL conical containing HPM.
7. Remove 1mL of this medium and hepatocyte suspension using a pipette and place into the vial, ensuring collection of any remaining cells.
8. Close the 50mL tube, ensure the cap is tightened, and invert the conical gently 3 to 4 times to ensure resuspension of the hepatocytes.
9. Centrifuge the conical at room temperature with the following rates depending on the plating media:
   a. HPM without density gradient: 100G x 5 minutes
   b. HPM with density gradient: 100G x 10 minutes
10. Remove the conical from the centrifuge.
11. Aspirate or pour off the supernatant.
12. Resuspend the cell pellet in 3 ~ 4mL of 37°C Hepatocyte Plating Media (HPM).
13. Proceed to the step 7 of the previous protocol: Thawing and plating hepatocytes without 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).

Figure 1. Image of hepatocytes after overnight incubation. Hepatocytes were plated in HPM overnight on collagen coated plates. Media was changed to maintenance medium before taking the images with Millicell® Digital Cell Imager at 10x (upper panel) or 20x (lower panel) magnification setting. Notice the hepatocytes show characteristic cobble stone morphology with well-defined borders.



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