

Human Hepatocytes

SKU:10HU-0233

PRODUCT SHEET

Product Description

The liver is a vital organ in mammals, and hepatocytes, the major cell type in liver, play critical roles in protein synthesis, detoxification of various metabolites, transformation of carbohydrates, and the production of biochemical necessary for digestion^[1]. Primary hepatocyte culture is an important model for in vitro studies in drug development, including hepatotoxicity, drug transport, hepatitis virus infection, hepatic drug metabolism and hepatobiliary excretion^[2].

Product Details

Catalog Number: 10HU-233

Organism: *Homo Sapiens*, Human

Cell Type: Liver Parenchymal Cells

Tissue: Human Liver

Package Size: 5.0 x 10⁶ cells/vial

Passage Number: P0

Growth Properties: Adherent on matrix coated dish

Associated Media: Human Hepatocyte Culture Kit (MD-0111)

Storage Conditions & Shipment

Product Format/Shipped: Cryopreserved

Storage: Liquid Nitrogen

Overview of Human Hepatocytes

iXCells Biotechnologies provides high quality human primary hepatocytes isolated from whole liver of human organ donors and cryopreserved on the day of isolation with ≥5 million viable cells in each vial. iXCells offers human hepatocytes in plateable & suspension categories and includes cells from both healthy and diseased tissue. Our cryoplateable primary human hepatocytes (CPHH) reach and maintain a confluent monolayer (>90% confluency) for 5-7 days (Fig 1), express general hepatocyte marker albumin, and show accumulation of lipid droplets (Fig 2). Each lot of human hepatocytes is QC tested & validated including key Cytochromes P450 (CYPs) enzyme activity and gene expression induction. They are negative for HIV-1/2, HBV, HCV, mycoplasma, bacteria, yeast, and fungi.

For Research Use Only

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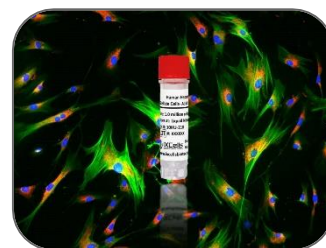
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RELATED PRODUCTS



iXCells™ Human Hepatocyte Culture Kit

SKU: MD-0111



Human Hepatic Stellate Cells - Adult (HHSC)

SKU: 10HU-210

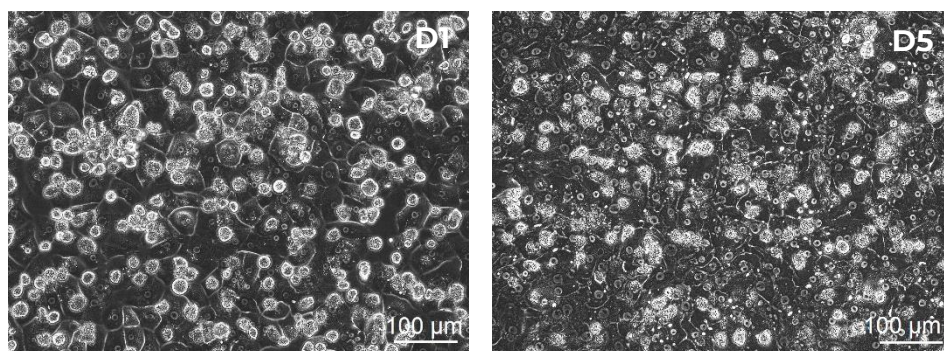


Figure 1. Phase contrast images of CPHH. CPHH were plated in a 6-well culture dish precoated with Collagen type I and cultured for 5 days following iXCells protocol. CPHH reached and maintained monolayer with >90% confluency. Hepatocytes exhibited hexagonal shape with single or double nuclei and characteristic “chicken wire” like bile canaliculus formation between hepatocytes.

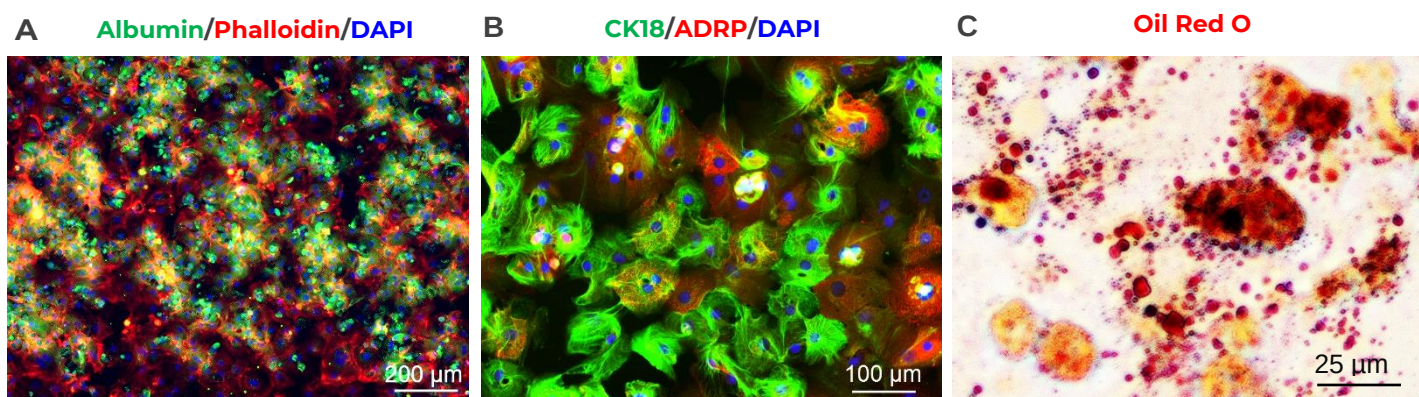


Figure 2. Immunostaining and Oil Red O staining of cultured CPHH at D5. CPHH were plated in a 12-well culture dish and cultured for 5 days. **(A)** Albumin recognized by anti-human albumin antibody (green), and cytoskeleton marker F actin revealed by Alexa Fluor 555 Phalloidin (red), with cell nuclei counterstained by DAPI (blue). **(B)** Hepatocyte cytoskeletons were stained with anti-Cytokeratin 18 (CK18, green) antibody and intracellular lipid droplets were recognized by anti-ADRP/Perilipin 2 antibody (red). **(C)** Lipid droplet staining using Oil red O in CPHH.

P450 CYP Enzyme Induction

Enzyme Isoform	Inducer	Assay	Enzymatic Fold Induction	Assay	mRNA Fold Induction
CYP1A2	50 μM Omeprazole	1A2 Glo™ Assay	4.59	TaqMan q-PCR	32.7
CYP3A4	25 μM Rifampin	3A4 Glo™ Assay	3.89	TaqMan q-PCR	41.6

Table 1. Representative data of inducible hepatocytes. Cryopreserved human hepatocytes were thawed, plated in a collagen Type 1 coated 12-well culture plate (750,000 cells/well). The hepatocytes were incubated with appropriate inducers or vehicle control (0.1% DMSO) for 48 hours. For P450 CYP enzymatic activity induction, Luciferin-1A2 or Luciferin-IPA substrate was added to the plate and CYP1A2 or CYP3A4 enzymatic activities was detected using P450-Glo™ Assay (Promega, Cat. No. V8421; V9001). The fold changes of the drug induced P450 CYP enzyme activity over the basal level were calculated. For CYP gene expression induction, hepatocyte RNA was extracted after inducer treatment, and the mRNA expression levels of CYP1A2 and CYP3A4 were analyzed using TaqMan qRT-PCR. The fold changes of the drug induced P450 CYP gene expression over the basal levels were calculated using the $\Delta\Delta C_T$ method.

QuickStart Guide – Protocols

Required reagents and materials

- iXCells™ Human Hepatocyte Culture Kit (Cat# MD-0111) includes:

Medium	CAT #	Volume
Human Hepatocyte Thawing Medium (HHTM)	MD-0111T	25 mL
Human Hepatocyte Plating Medium (HHPM)	MD-0111P	10 mL
Human Hepatocyte Maintenance Medium (HHMM)	MD-0111M	100 mL

Store the HHTM, HHPM and HHMM at -20°C for 12 months, or 1 month at 4 °C. The kit is for ONE cryovial.

Other required reagents:

- 0.1% Solution of Trypan Blue
- Collagen, Type I solution from rat tail
- Optional: Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix for overlaying plated hepatocytes

Coating Culture Vessel

- Dilute Collagen Type I solution from rat tail (Sigma-Aldrich, C3867) with 1× DPBS to prepare final concentration of 0.1 mg/mL.
- Coat the surface(s) of culture vessels with necessary volume of collagen solution. For example, 1-2 mL of the above solution for one well of a 6-well plate, and 0.5-1.0 mL for one well of a 12-well plate.
- Incubate at room temperature for one hour or 4 °C overnight.
- Carefully aspire the remaining solution and use 1× DPBS to rinse the dishes/plates three times. Use the coated dishes/plated immediately or store them at 2-8 °C and use them within 1 week.

Thawing and plating of Cryoplateable Primary Human Hepatocytes (CPHH)

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

- Warm **Human Hepatocyte Thawing Medium (HHTM)** and **Human Hepatocyte Plating Medium (HHPM)** to 37 ± 1°C in water bath and put them in a laminar hood before use. Take 20 mL HHTM into a 50 mL centrifuge tube.
- Remove **Human Hepatocyte (Cat# 10HU-233)** cryotube from the LN₂ storage unit and immediately place in a 37°C water bath for 90~120 seconds until the frozen cell pellet can move freely when the cryotube is inverted (Do not over-thaw).

3. Wipe the cryotube with 70% ethanol. Pour the frozen pellet from the cryotube into the centrifuge tube containing 20 mL **HHTM**. Rinse the cryotube with 1.0 mL of **HHTM**. Combine this rinse with the cells and gently invert until fully melted.
4. Centrifuge the cells at 100g × 5 minutes at room temperature. Carefully aspirate and discard the supernatant without disturbing the cell pellet.
5. Dislodge the cell pellet from the tube wall by agitating the tube gently, then resuspend the cell pellet with 5 mL prewarmed **Human Hepatocyte Plating Medium (HHPM)** using the 5 mL serological pipette.
Note: Do not over-dilute the cells based on final target cell concentration. If the cells are advertently over-diluted, spin down the cells at 80g × 5 minutes, and resuspend the cell pellet in desired volume of HHPM.
6. Mix 20 µL of the homogenous cell suspension with 20 µL **0.1% Trypan Blue Solution** by gently pipetting up and down 3-5 times, and perform manual cell counting using the hemocytometer.
Note: Do not vortex the cells and trypan blue solution.
Note: The automated cell counter is not recommended.
7. Seed the cells on the precoated culture vessels with **Human Hepatocyte Plating Medium (HHPM)** at the desired cell seeding density (see LOT CoA for recommended seeding density).
Note: The cryopreserved hepatocytes from certain donor may not adhere well to 96-well plates and may require wells with a larger surface area. Please find the recommended culture condition in respective LOT CoA.

Table 1. Recommended seeding density of human hepatocytes

Plate Format	Seeding Density (Million cells/mL)	Feeding Volume Per Well	Total Cells (Cells/Well)
6-Well	0.75-1.5	2 mL	1.5-3.0 million
12-Well	0.75-1.5	0.8 mL	0.6-1.2 million
24-Well	0.75-1.5	0.5 mL	375,000-750,000
48-Well	0.75-1.5	200 µL	150,000-300,000
96-Well	0.75-1.5	100 µL	75,000-150,000

Note: The recommended initial seeding density is based on the recovery tests performed in house on the LOT during post-cryopreservation quality control. Please see CoA for details.

8. Place the cells in the incubator at 37°C with 5% CO₂. Move the culture plates in a “figure-eight” movement every 15 minutes for 2-3 times, to achieve the even cell distribution.
Note: For 96-well plate, please do not shake.

9. Allow the cells to attach for an additional ~4 hours in the incubator without disturbing. Gently aspirate media to remove non-attached cells. Add prewarmed **Human Hepatocyte Maintenance Medium (HHMM)** slowly to the attached cells and culture for further 12-16 hours. Experiments can start after this time point.
10. Replace prewarmed **Human Hepatocyte Maintenance Medium (HHMM)** daily. HHMM maintains stable, confluent monolayers and structure integrity for up to 7 days.

For overlay of Matrigel (Optional):

Once the plateable hepatocytes are adhered to the surface of the culture plate, swirl the culture vessel to suspend the unattached cells and gently aspirate media containing non-attached cells. Do NOT over dry the cells.

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix solution is stored at -20°C. It is recommended to thaw frozen Matrigel stock in the refrigerator the day prior to use. Place Matrigel stock solution and **Human Hepatocyte Maintenance Medium (HHMM, Cat# MD-0111M)** on ice. Calculate the volume of maintenance medium needed to overlay the plated hepatocytes, using volumes shown in Table 2. We recommend overlaying Matrigel concentration at 3.0 mg/mL Mix Matrigel with **HHMM** well by pipetting several times and add chilly cold **Matrigel-HHMM Solution** to each well or plate to overlay the attached cells. Return the plates to the incubator at 37°C with 5% CO₂. Apply Matrigel-HHMM Solution to plated hepatocytes at least for 12 hours before starting experiments.

Table 2. Human hepatocyte maintenance medium volume per well

Plate/Dish Format	Volume Per Well for Media Change
6-Well	2 mL
12-Well	1 mL
24-Well	0.5 mL
48-Well	200 µL
96-Well	100 µL
35 mm Plate	2 mL

References

- [1] Schulze RJ, Schott MB, Casey CA, Tuma PL, McNiven MA. The cell biology of the hepatocyte: A membrane trafficking machine. J Cell Biol. 2019 Jul 1;218(7):2096-2112.
- [2] Zeilinger K, Freyer N, Damm G, Seehofer D, Knöspel F. Cell sources for in vitro human liver cell culture models. Exp Biol Med(Maywood). 2016 Sep;241(15):1684-98.

Disclaimers

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