www.ixcellsbiotech.com
7270 Trade Street, Suite 102, San Diego, CA 92121
Tel: (858) 412-5988 Fax: (858)368-8716
Technical Supports: supports@ixcellsbiotech.com
Orders: orders@ixcellsbiotech.com

Product Information

Human Hepatocytes

Catalog Number	10HU-233	**Cell Number**	5 million cells/vial
Species	*Homo sapiens*	**Storage Temperature**	Liquid Nitrogen

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].

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 cells in each vial. Our cryoplateable primary human hepatocytes (CPHH) reach and maintain a confluent monolayer (>85% confluency) for at least 7 days, express general hepatocyte marker albumin, and show accumulation of lipid droplets. Each lot of human hepatocytes is QC tested & validated for key Cytochromes P450 (CYPs) enzyme activity induction. They are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. CPHH cannot be subcultured or passaged.

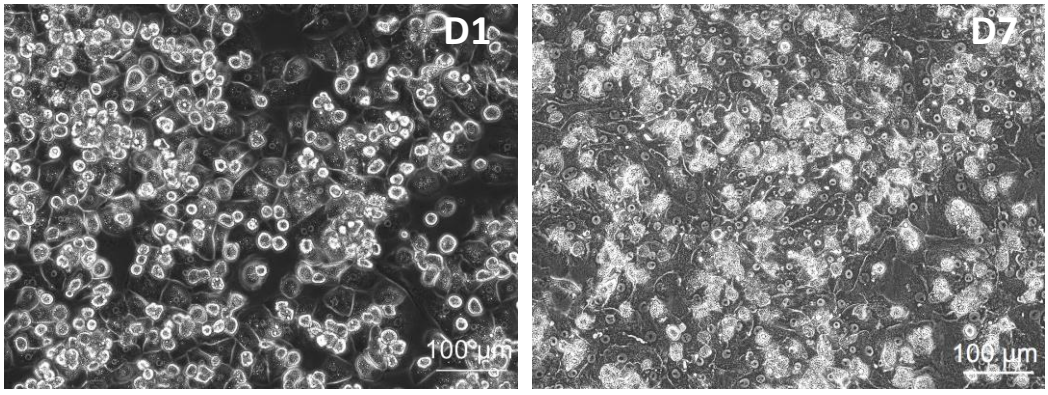

Figure 1. Phase contrast images of CPHH. CPHH were plated in a 6-well culture dish (2 million cells/well) and cultured for 7 days. CPHH reached and maintained monolayer with >90% confluency. Hepatocytes exhibited hexagonal shape with single or double nuclei and characteristic “chicken wire” like bile canaliculus formation between hepatocytes.

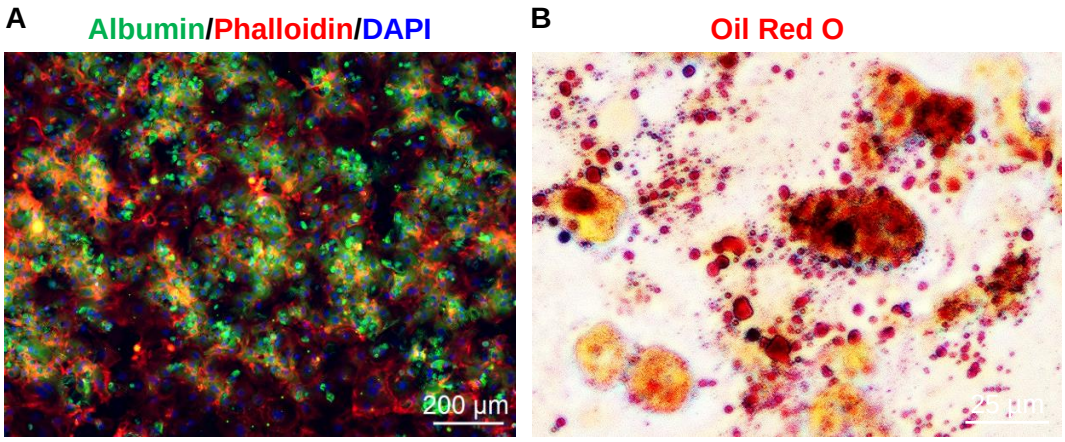

Figure 2. Immunostaining and Oil Red O staining of cultured CPHH at D5. CPHH were plated in a 12-well culture dish (750,000 cells/well) and cultured for 5 days. **(A)** Albumin recognized by anti-human albumin antibody (green), and cytoskeleton marker F actin revealed by Alexa Fluor 555 Phalloidin (red), with cell nuclei counterstained by DAPI (blue). **(B)** Lipid droplet staining using Oil red O in CPHH.

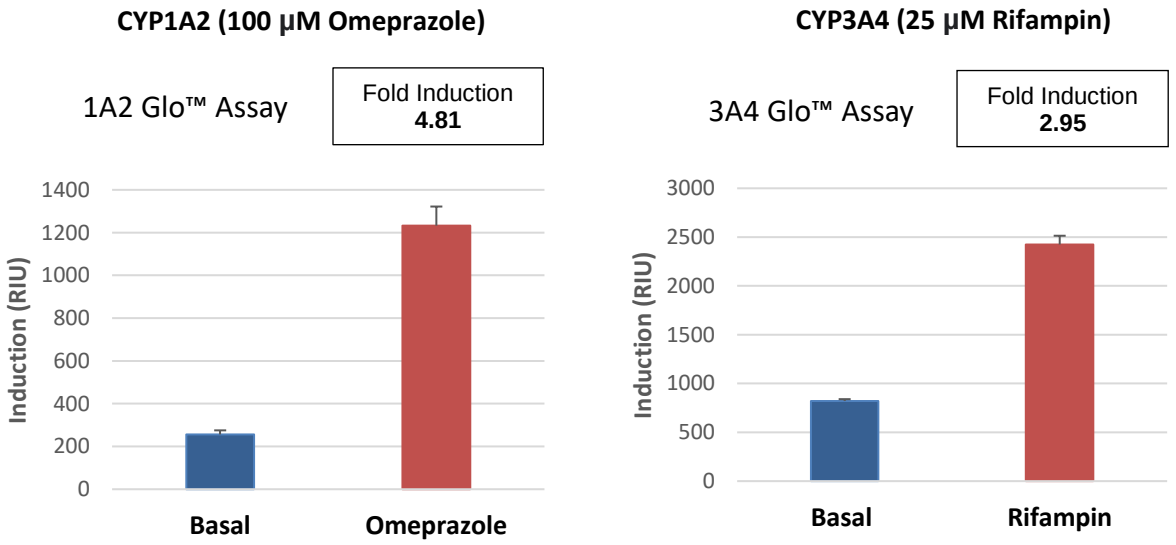

Figure 3. P450 CYP activities induced by Omeprazole or Rifampin. CPHH were plated in a 12-well culture dish (750,000 cells /well) and incubated with appropriate inducers for 48 hours. Supernatant from induced and uninduced cells were analyzed using P450-Glo™ Assay (Promega, Cat. No. V8421; V8911). The fold changes of the drug induced P450 CYP enzyme activity over the basal level were calculated.

Product Details

Tissue	Human liver
Package Size	5 million cells/vial
Passage Number	P0-plateable
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	iXCells™ Human Hepatocyte Culture Kit (Cat# MD-0111)

Protocols

This procedure describes the steps required for thawing and plating CPHH using iXCells™ Human Hepatocyte Culture Kit (Cat# MD-0111).

Required reagents and materials

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

		Volume
Human Hepatocyte Thawing Medium A (HHTM-A)	MD-0111A	35 mL
Human Hepatocyte Thawing Medium B (HHTM-B)	MD-0111B	15 mL
Human Hepatocyte Plating Medium (HHPM)	MD-0111P	25 mL
Human Hepatocyte Maintenance Medium (HHMM)	MD-0111M	100 mL

Store the HHTM-A and HHTM-B at 2-8ºC for 3 months, and the HHPM and HHMM at -20ºC for 6 months. 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 of Culture Vessels

1. Dilute Collagen Type I solution from rat tail (Sigma-Aldrich, C3867) with 1× PBS to prepare final concentration of 0.1 mg/mL.
2. 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 mL for one well of a 12-well plate.
3. Incubate at room temperature for one hour or 4° C overnight.
4. Carefully aspire the remaining solution and use 1× PBS 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.*

1. Warm **Human Hepatocyte Thawing Medium A (HHTM-A)** and **Human Hepatocyte Plating Medium (HHPM)** to 37 ± 1°C in water bath (usually take 15-20 minutes) and put them in a laminar hood before use. Take 34 mL HHTM-A into a 50 mL centrifuge tube. Place **Human Hepatocyte Thawing Medium B (HHTM-B)** in a laminar hood before thawing hepatocytes.
2. Remove **Human Hepatocyte (Cat# 10HU-233)** cryotube from the LN_2 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 34 mL **HHTM-A**. Rinse the cryotube with 1.0 mL of **HHTM-A**. Combine this rinse with the cells and gently invert until fully melted. Transfer 15 mL **HHTM-B** to the tube containing the cells. Mix well by inverting the tube a few times. Do not vortex or shake.
4. Centrifuge the cells at 168*g* × 20 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 10 mL Prewarmed **Human Hepatocyte Plating Medium (HHPM)**. Be careful not to over-dilute the cells based on final target cell concentration. *If the cells are advertently over-diluted, spin down the cells at 80*g* × 5 minutes, and resuspend the cell pellet in desired volume of HHPM.
6. Remove 50 µL of the homogenous cell suspension and dispense the 50 µL **0.1% Trypan Blue Solution**, aliquot into the Cell Count tube. Mix gently the hepatocytes and the Trypan Blue Solution (do not vortex).
7. Add an aliquot of 20 µL Trypan Blue Solution and cells mixture into a hemacytometer and count viable cells manually. Do not use automated cell counter.
8. After counting, seed the cells on the coated culture dishes/plates with **Human Hepatocyte Plating Medium (HHPM)** with the desired cell seeding density as recommended in Table 1. Note that some cryopreserved hepatocytes may not adhere well to 96-well plates and may require wells with a larger surface area.

Table 1. Recommended seeding density of human hepatocytes

Plate Format	Seeding Density (million cells/mL)	Feeding Volume Per Well
6-Well	1.0	2 mL
12-Well	0.75	1 mL
24-Well	0.75	0.5 mL
48-Well	0.75	200 µL
96-Well	0.60	100 µL

9. Place the cells in the incubator at 37°C with 5% CO_2. Once the plates are placed in an incubator, move the culture plates in a "T" or "figure-eight" movement, except for the 96-well plates that should NOT be shaken.
10. During the first half hour after the seeding, use "figure-eight" movement every 15 minutes. Check the cells under microscope until an equal cell distribution is achieved.
11. Let the cells attach for at least 3-4 hours in the incubator. Gently aspirate media containing non-attached cells. Do not disrupt the attached cells. Add fresh hepatocyte plating medium **(HHPM)** to the attached cells and culture for additional 12-16 hours.
12. <u>For overlay of Matrigel:</u>

    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 a 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 overlay Matrigel concentration at 3 mg/mL. Mix Matrigel with **HHMM** well by pipetting serval 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% CO_2. Apply Matrigel-HHMM Solution to plated hepatocytes at least for 12 hours before starting experiments.

13. <u>For "no-overlay"</u>

    Replace prewarmed **Human Hepatocyte Maintenance Medium (HHMM)** daily. HHMM maintains stable, confluent monolayers and structure integrity for up to 7 days.

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

Reference

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

This product is intended for laboratory research purposes only. It is not intended for use in humans. While iXCells Biotechnologies uses reasonable efforts to include accurate and up-to-date information on this product sheet, we make no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. iXCells Biotechnologies does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. iXCells Biotechnologies is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of strains on deposit, iXCells Biotechnologies is not liable for damages arising from the misidentification or misrepresentation of cultures.