

**HHCP-I-3D Human Hepatocytes Cryopreserved Plateable for induction assays and for 3D culture**  
**Cell Specification – Certificate of Analysis (CoA)**

Lot cPHH060625485

Batch Release: March 30, 2026

**Donor data**

Species: Human

Gender: female

Age: 38 years

Smoker: no

Diagnosis: Biliary colic and angiomyolipoma in left lateral liver  
Medical History: no

Therapy: Hemihepatectomy left

Medication: no

Serology: negative for HBV, HCV, HIV 1/2

**Cryopreservation and Thawing**

**Cryopreservation:**

Date: June 6, 2025

Amount per vial:  $10.0 \times 10^6$  cells

**Thawing:** n=1

Post-thaw viability: 97.4 %

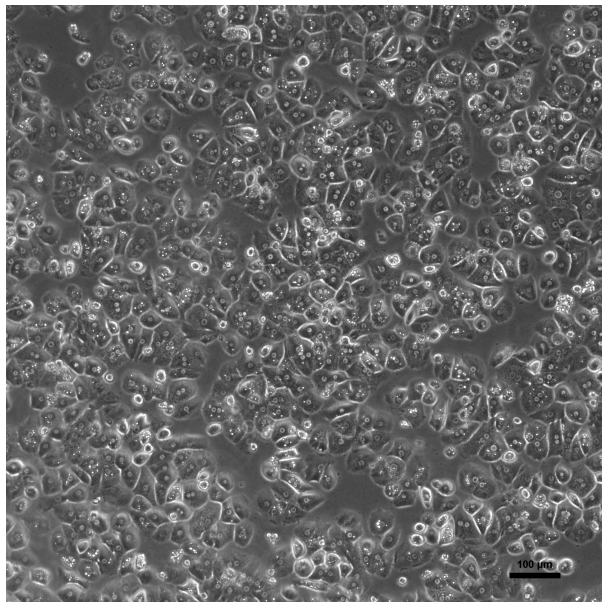
Post-thaw yield per vial:  $3.4 \times 10^6$  cells

Recovery: 34 %

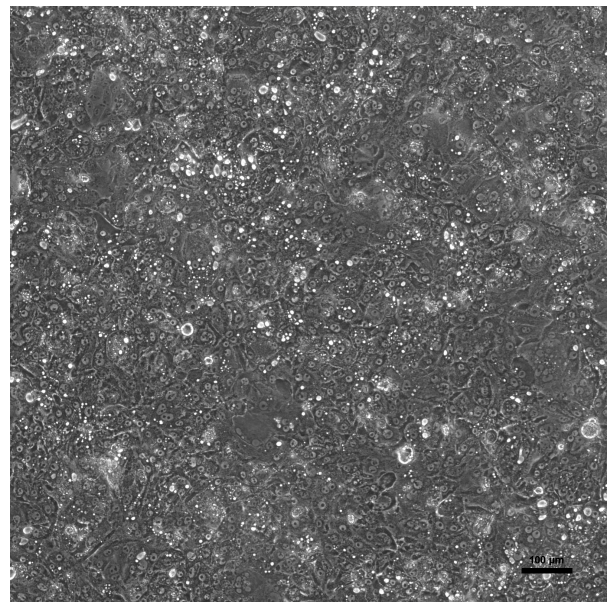
**Only one spin required. No washing step.**

**2D culture**

Phase contrast on day 1 after thawing  
(24well plate)



Phase contrast on day 4 after thawing  
(24well plate)



Recommended seeding density on collagen-coated plates:  
24well plate – 300,000 cells/well // 96well plate – 80,000 cells/well,  
Culture in Human Hepatocyte Maintenance Medium (HHMM).

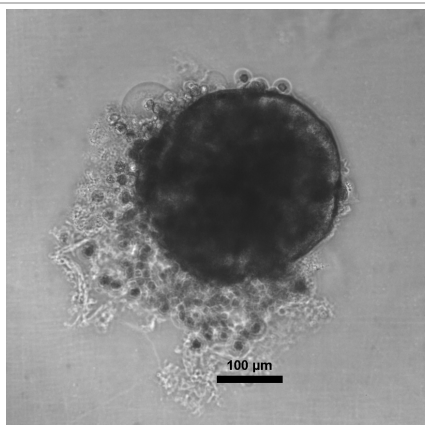
**Note:** Gently shake the plate (N/S-E/W) every 30 minutes for 2 hours after plating (only 24well plate and bigger wells). This step has a positive effect on the uniform plating.

CYP P450 activity in 2D culture after thawing:	pmol/(mg × min)	X-fold induction
Ethoxyresorufin-O-deethylation:	24well: 10.7 ± 0.3	3.2
Induction with 25 µM β-Naphthoflavone for 3 d	96well: 16.4 ± 2.0	4.4

### 3D culture

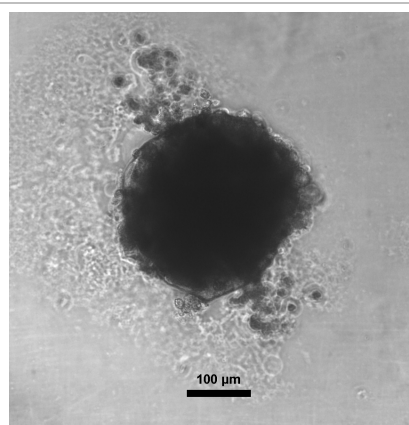
Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well

day 7



scale bar 100 µm

day 11



scale bar 100 µm

### Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10<sup>6</sup> cells in 0.5 mL HPM-Cryo):

Time (h)	0	1	2	3	4	5
Viability (%)	97.4	92.3	94.7	90.8	92.9	92.2

Note: Yield, viability, recovery and activity assays were performed at PRIMACYT using PRIMACYT's manual for thawing, plating and culture of primary cryopreserved hepatocytes.

**Note for thawing process: Only one spin at 100 x g, 10 min., 20 °C is required. No washing step needed.**

**Store at -150 °C or in the vapour phase of LN<sub>2</sub>**

This product should be considered as potential biohazard. Only intended for *in vitro* use.

Issued by: A. Ullrich

Verified by: J. Schuldt