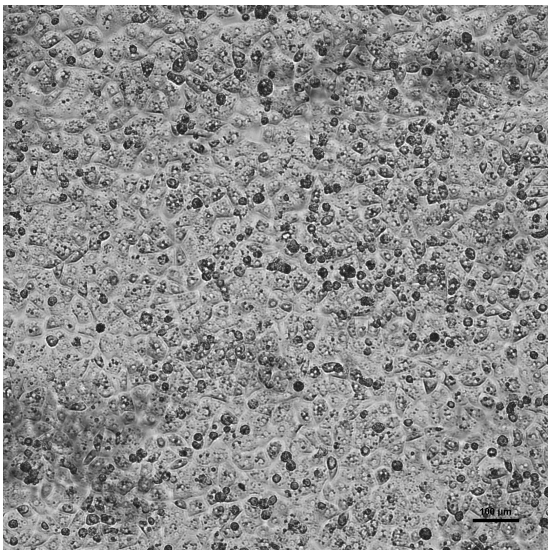
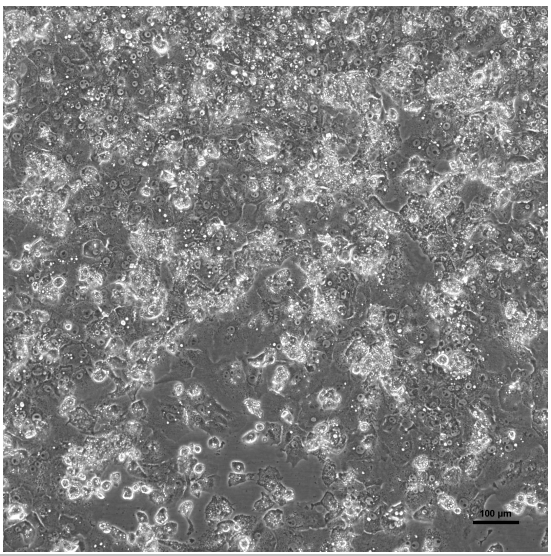
PRIMACYT
Cell Culture Technology

HHCP-I-3D Human Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture **Cell Specification – Certificate of Analysis (CoA)**	
Lot HH250606	Batch Release: August 01, 2025

Donor data	
Species: Human	Gender: male Age: 70 years
Diagnosis: suspected liver metastasis in renal cell carcinoma Medical History: Hypertension, Diabetes	Therapy: Liver resection left Medication: ASS, Candesartan, Atorvastatin, Pantoprazol, Sitagliptin, Empagliflozin, Mirtazapin, Tricylglycerollipase Serology: HAV, HBV, HCV, HIV 1/2: negative

Cryopreservation and Thawing	
Cryopreservation: Date: June 06, 2025 Amount per vial: 8.1 x 10^6 cells	**Thawing:** n=1 Post-thaw viability: 94.4 % Post-thaw yield per vial: 2.77 x 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 3 after thawing (24well plate)
	

E-mail: info@primacyt.de, Tel.: +49 (0) 385-3993-600, Fax: +49 (0) 385-3993-602

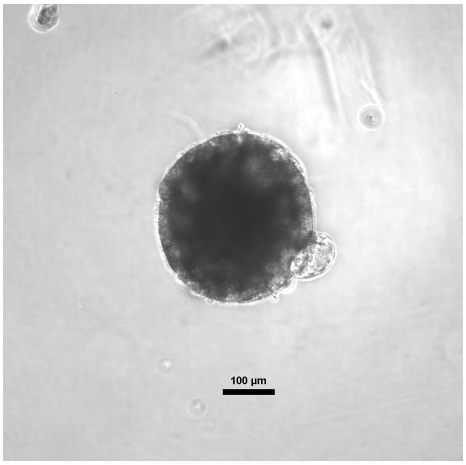
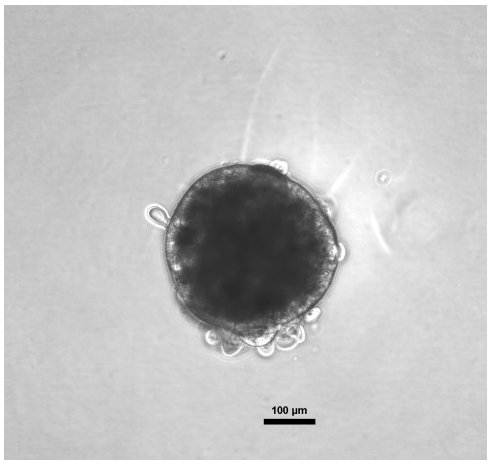
PRIMACYT
Cell Culture Technology

Recommended seeding density on collagen-coated plates:
24well plate – 400,000 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: 92.2 ± 1.5	3.9
Induction with 25 µM ß-Naphthoflavone	96well: 77.8 ± 4.7	4.5

3D culture

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

day 7	day 10
	
scale bar 100 µm	scale bar 100 µm

Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10^6 cells in 0.5 ml HPM-Cryo):

Time (h)	0	1	2	3	4	5
Viability (%)	94.4	90.0	85.5	88.2	87.7	86.6

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_2

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

Issued by: M. Reu	Verified by: K. Damrau