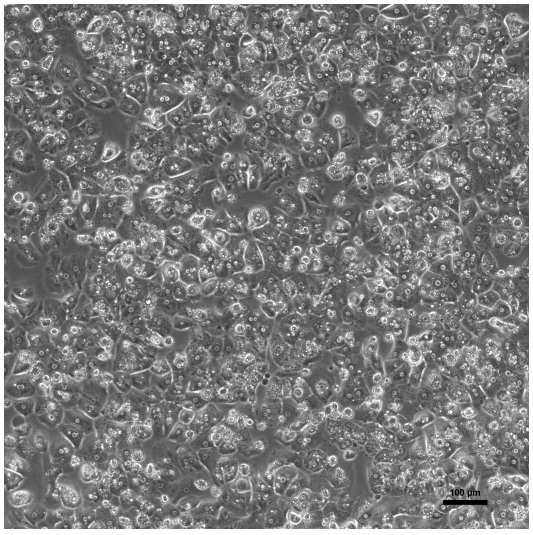
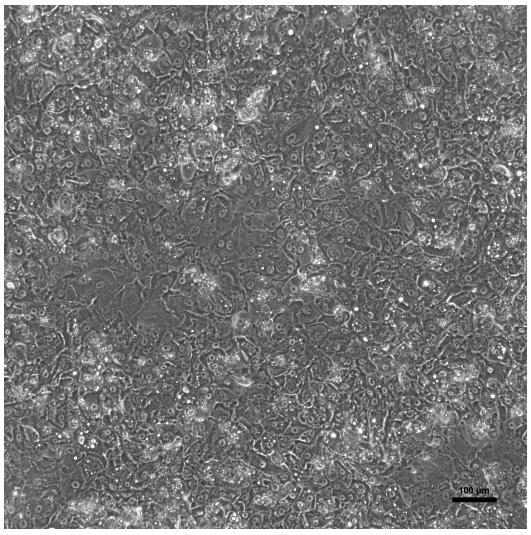
PRIMACYT
Cell Culture Technology

HHCP-I-3D Human Hepatocytes Cryopreserved Plateable for induction assays and for 3D culture **Cell Specification – Certificate of Analysis (CoA)**	
Lot cPHH200625488	Batch Release: March 30, 2026

Donor data	
Species: Human	Gender: male Age: 63 years Smoker: no
Diagnosis: Colorectal liver and lung metastases Medical History: Caecal cancer treated with laparoscopic right hemicolectomy and adjuvant chemotherapy (CAPOX – Capecitabin and Oxaliplatin, 4 weekly, 3 cycles).	Therapy: Open extended right hemi-hepatectomy followed by lung ablation Medication: no Chemotherapy: FOLFIRI (Folinic acid), Fluorouracil (5-FU) and Irinotecan, 6 cycles Serology: negative for HBV, HCV, HIV 1/2

Cryopreservation and Thawing	
Cryopreservation: Date: June 20, 2025 Amount per vial: 10.0 x 10^6 cells	**Thawing:** n=1 Post-thaw viability: 95.2 % Post-thaw yield per vial: 6.5 x 10^6 cells Recovery: 65 % **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

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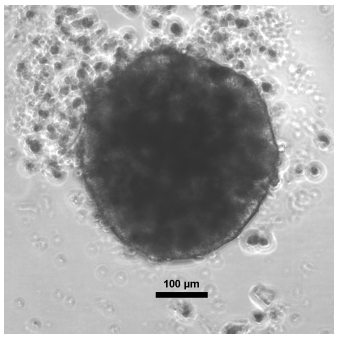
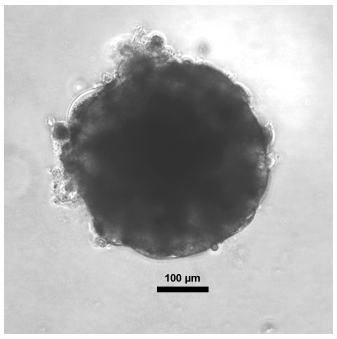
Recommended seeding density on collagen-coated plates:
24well plate – 300,000 cells/well // 96well plate – 70,000 cells/well,
Culture in Human Hepatocyte Maintenance Medium (HHMM).

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: 5.8 ± 0.2	3.0
Induction with 25 µM ß-Naphthoflavone for 3 d	96well: 8.6 ± 2.2	3.6

3D culture

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

day 4	day 9
	
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 (%)	95.2	88.6	92.6	91.0	90.1	87.3

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: A. Ullrich	Verified by: J. Schuldt