

HHCP-I-3D Human Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture

Cell Specification – Certificate of Analysis (CoA)

Lot HH250220

Batch Release: August 01, 2025

Donor data

Species: Human

Gender: male

Age: 85 years

Diagnosis: CCC (Cholangiocellular carcinoma)
Medical History: Diabetes, Hypertension

Therapy: Hemihepatectomy right
Medication: Apixaban, Entresto, Forxiga,
Bisoprolol, Torasemid, Atorvastatin,
Pantoprazol, Tamsulosin, Eisen, Symbicort,
Torem
Serology: HAV, HBV, HCV, HIV 1/2: negative

Cryopreservation and Thawing

Cryopreservation:

Date: Feb 25, 2025

Amount per vial: 5.14×10^6 cells

Thawing: n=1

Post-thaw viability: 91.3 %

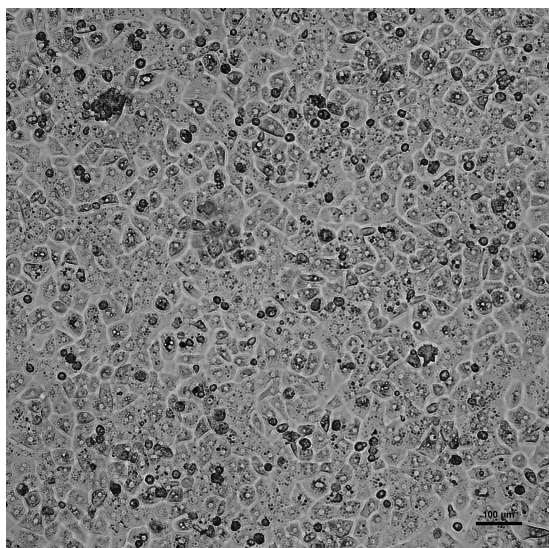
Post-thaw yield per vial: 1.8×10^6 cells

Recovery: 35 %

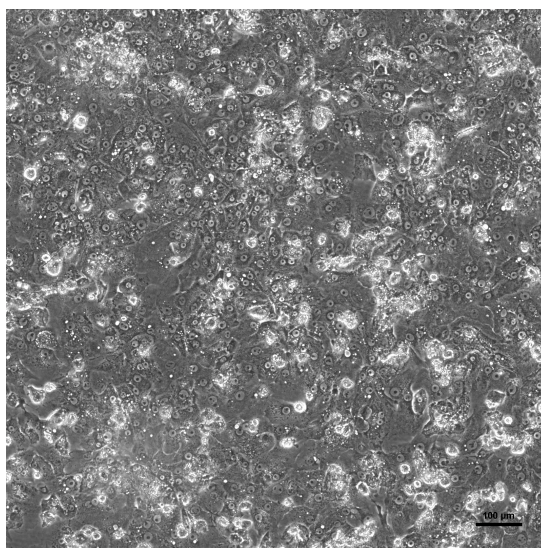
Only one spin required. No washing step.

2D culture

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



Phase contrast on day 3 after thawing
(24well plate)



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).

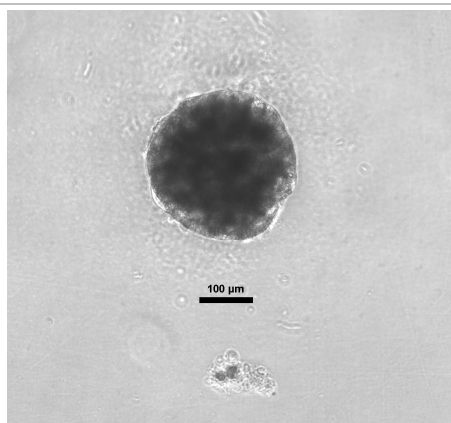
Note: Gently shake the plate (N/S-E/W) every 30 minutes for 2 hours after plating (only 24well plate and bigger wells). This step has a positive effect on the uniform plating.

CYP P450 activity in 2D culture after thawing:	pmol/(mg × min)	X-fold induction
Ethoxyresorufin-O-deethylation:	96well: 83.0 ± 8.4	2.7
Induction with 25 µM β-Naphthoflavone		

3D culture

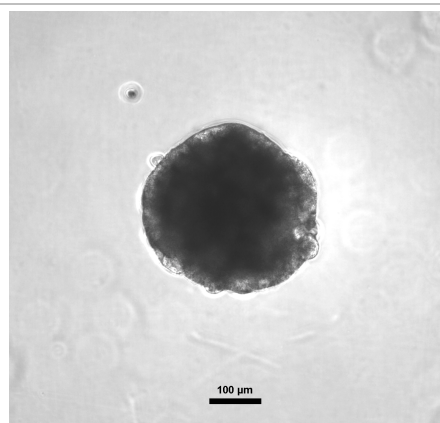
Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well

day 7



scale bar 100 µm

day 10



scale bar 100 µm

Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10⁶ cells in 0.5 ml HPM-Cryo):

Time (h)	0	1	2	3	4	5
Viability (%)	91.3	87.8	76.8	85.1	75.4	79.9

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 LN₂

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

Issued by: M. Reu

Verified by: K. Damrau

