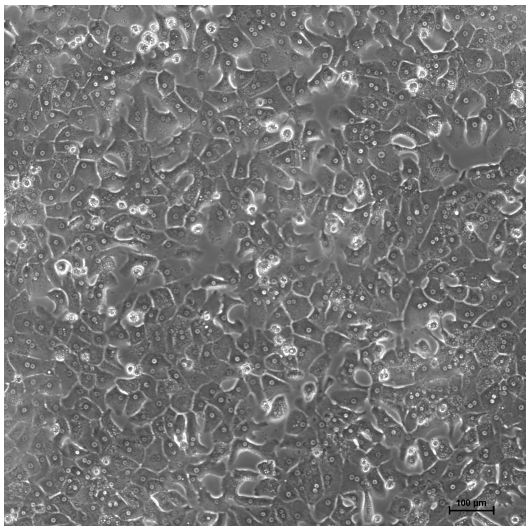
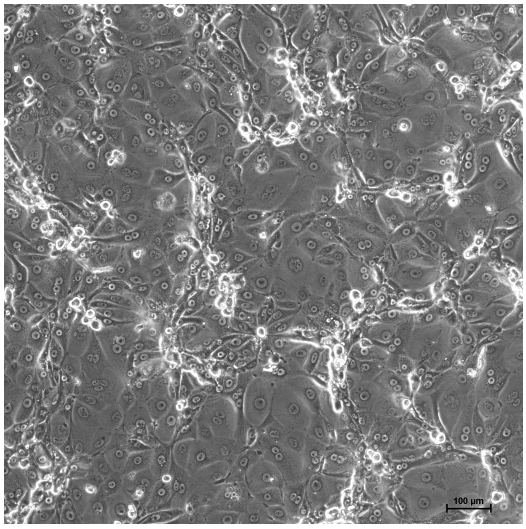
PRIMACYT
Cell Culture Technology

BHCP-I-3D Beagle Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture **Cell Specification – Certificate of Analysis (CoA)**	
Lot BH180829	Batch Release: Nov 23, 2017 Update: Oct 29, 2024

Donor data	
Species: Beagle	Gender: male Age: 7.5 months

Cryopreservation and Thawing	
Cryopreservation: Date: Aug 29, 2018 Amount per vial: 10.5 x 10^6 cells	**Thawing:** n=6 Post-thaw viability: 90.8 ± 1.5 % Post-thaw yield per vial: 6.1 ± 1.6 x 10^6 cells Recovery: 58 ± 15 %

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:
12well plate – 800,000 cells/well // 24well plate – 400,000 cells/well //
96well plate – 90,000 cells/well.
Culture in Human Hepatocyte Maintenance Medium (HHMM).

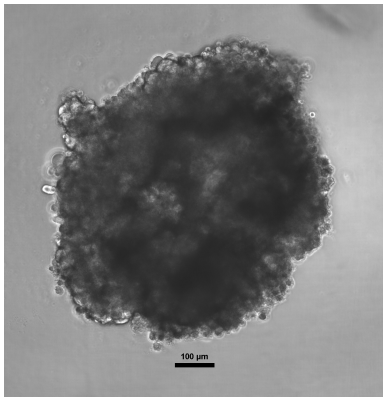
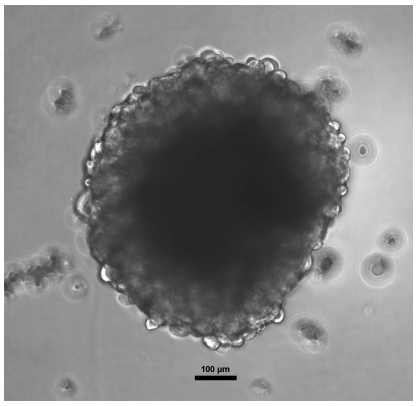
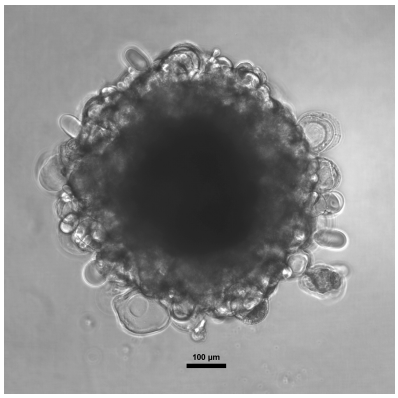
CYP P450 activity in 2D culture after thawing:	pmol/(mg × min)	X-fold induction
Ethoxyresorufin-O-deethylation: Induction with 25 µM ß-Naphthoflavone	24well: 107.9 ± 5.8 96well: 198.9 ± 16.7	13.2 18.0

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3D culture

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

day 3	day 7	day 10
		

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	0.5	1	1.5	2	3	4	5
Viability (%)	92.7	84.2	90.5	87.8	85.4	92.0	73.9	80.0

Determination of CYP activities in suspension (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10^6 cells in 0.5 ml HPM Cryo medium with 5 % FCS):

Assay	**Enzyme activities (pmol/min*mg protein)** mean ± SD
Phenacetin-O-deethylase	70.3 ± 1.4
Bupropion-hydroxylase	21.7 ± 0.6
Midazolam 1'-hydroxylase	47.8 ± 3.8
UDP-Glucuronosyltransferase	180.1 ± 3.8
Sulfotransferase	44.5 ± 1.2

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

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: K. Damrau