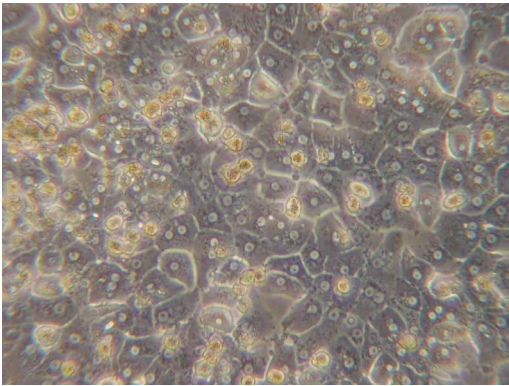
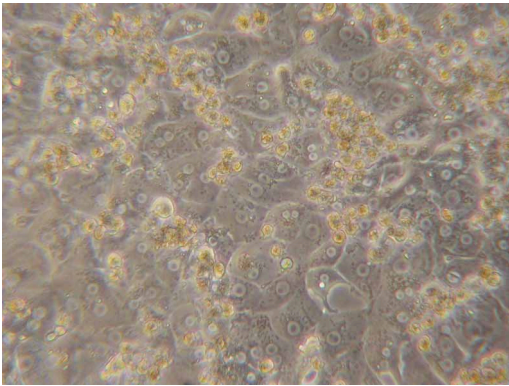


BHCP-I-T Cryopreserved Plateable Beagle Hepatocytes for Induction and Transporter assays
Cell Specification

Lot BH140616-1	Batch Release: November 06, 2014
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Species: Beagle	Gender: female	
	Age: 6 years 5 months	
Cryopreservation: Date: June 16, 2014 Amount per vial: 10 x 10 ⁶ cells	Thawing: Post-thaw viability: 75 % Post-thaw yield per vial: 4.8 x 10 ⁶ cells Recovery: 48 %	
Phase contrast on day 1 after thawing 	Phase contrast on day 2 after thawing 	
Recommended seeding density on collagen-coated plates: 283,000 cells per cm ² Culture in Human Hepatocyte Maintenance Medium (HHMM).		
CYP P450 activity in culture after thawing:	pmol/(mg × min)	x-fold induction
Ethoxyresorufin-O-deethylation:		
Induction with 10 µM beta-naphthoflavone	1.97 ± 0.28	12.3
Induction with 25 µM beta-naphthoflavone	3.16 ± 0.1	19.8

Uptake transporters: uptake of 10 µM Estrone 3-sulfate (E ₃ S) with or without competitive inhibitor Bromosulphophthalein (BSP, 100 µM) in cryopreserved hepatocytes after 2 min incubation.		
Activity of uptake transporters in culture after thawing	intracellular E ₃ S (pmol/mg × min)	Inhibition (%)
without BSP	195 ± 54	
with BSP	105 ± 12	46.1

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