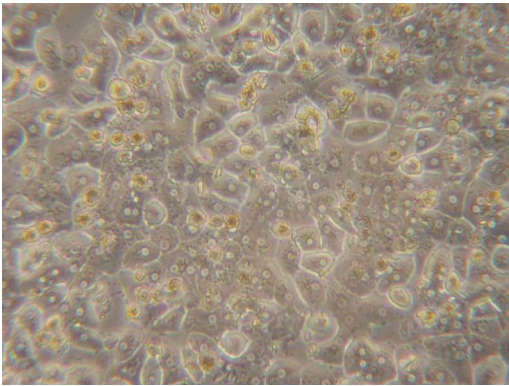
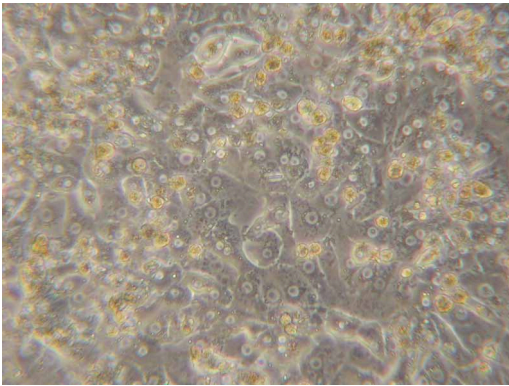


BHCP-I-T Cryopreserved Plateable Beagle Hepatocytes for Induction and Transporter assays
Cell Specification

Lot BH140616-2	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: 10.3 x 10 ⁶ cells	Thawing: Post-thaw viability: 66 % Post-thaw yield per vial: 4.6 x 10 ⁶ cells Recovery: 44.7 %				
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: Ethoxyresorufin-O-deethylation: Induction with 25 µM beta-naphthoflavone	<table border="1"> <tr> <td>pmol/(mg × min)</td> <td>x-fold induction</td> </tr> <tr> <td>4.09 ± 0.67</td> <td>19.6</td> </tr> </table>	pmol/(mg × min)	x-fold induction	4.09 ± 0.67	19.6
pmol/(mg × min)	x-fold induction				
4.09 ± 0.67	19.6				

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	163 ± 4	
with BSP	94 ± 26	42.3

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