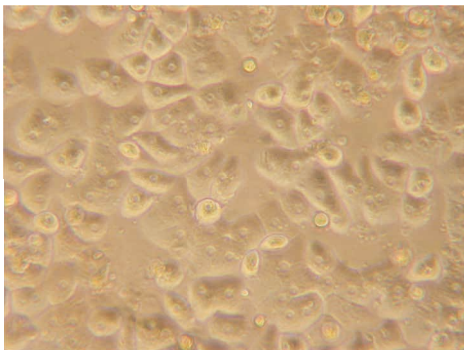
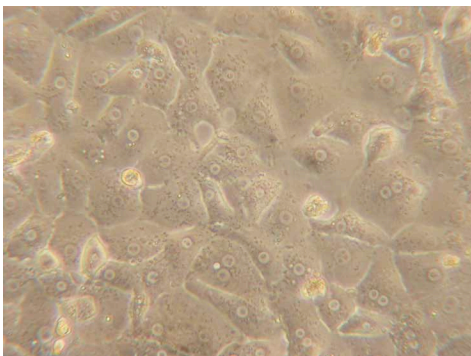


BHCP-I-T Cryopreserved Plateable Beagle Hepatocytes for Induction and Transporter assays
Cell Specification

Lot BH130510-1-1	Batch Release: November 06, 2014
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Species: Beagle	Gender: male						
	Age: 9 months						
Cryopreservation: Date: May 10, 2013 Amount per vial: 10.4 x 10 ⁶ cells	Thawing: Post-thaw viability: 71.8 % Post-thaw yield per vial: 7 x 10 ⁶ cells Recovery: 67 %						
Phase contrast on day 1 after thawing 	Phase contrast on day 3 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 10 µM beta-naphthoflavone Induction with 25 µM beta-naphthoflavone	<table border="1"> <thead> <tr> <th>pmol/(mg × min)</th> <th>x-fold induction</th> </tr> </thead> <tbody> <tr> <td>14.2 ± 1.6</td> <td>8.8</td> </tr> <tr> <td>37.5 ± 5.2</td> <td>23.4</td> </tr> </tbody> </table>	pmol/(mg × min)	x-fold induction	14.2 ± 1.6	8.8	37.5 ± 5.2	23.4
pmol/(mg × min)	x-fold induction						
14.2 ± 1.6	8.8						
37.5 ± 5.2	23.4						

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	229 ± 7	
with BSP	96 ± 2	58.1

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