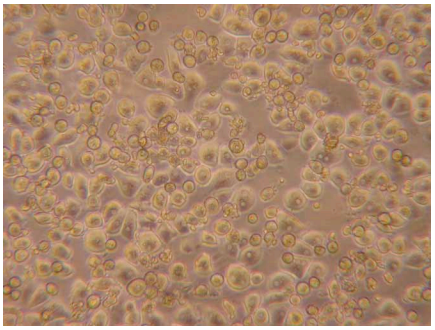
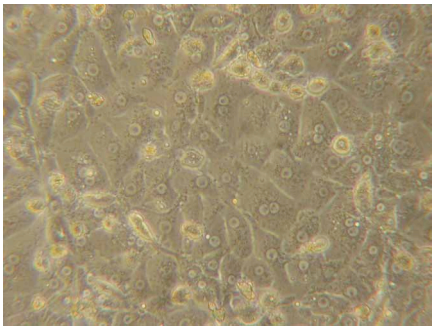
PRIMACYT
Cell Culture Technology

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

Lot BH130226	Batch Release: May 27, 2014

Species: Beagle	Gender: female Age: 1 year 3 months
Cryopreservation: Date: February 26, 2013 Amount per vial: 10 x 10^6 cells	Thawing: Post-thaw viability: 71 % Post-thaw yield per vial: 7.9 x 10^6 cells Recovery: 79 %
Phase contrast on day 0 after thawing	Phase contrast on day 3 after thawing

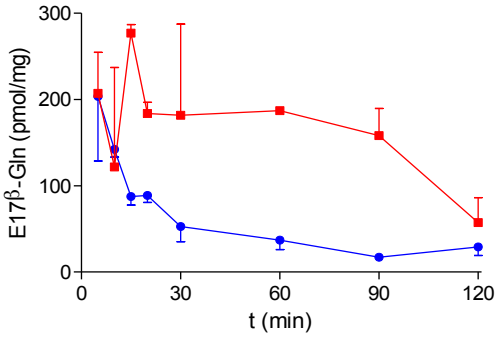




Recommended seeding density on collagen-coated plates: 283,000 cells per cm^2
Culture in Human Hepatocyte Maintenance Medium (HHMM).

CYP P450 activity in culture after thawing:	pmol/(mg × min)	x-fold induction
Ethoxyresorufin-O-deethylation:		
Induction with 10 µM beta-naphthoflavone	21.7 ± 4.0	20.9
Induction with 25 µM beta-naphthoflavone	48.8 ± 19.6	46.7

Efflux Transporter: after 5 min preincubation with 10 µM Estradiol-17-beta-glucuronide (E17ß-Gln), Multidrug resistance-related protein 2 (Mrp2) mediated Efflux of E17ß-Gln with or without the specific Mrp2 inhibitor MK571 (10 µM) in cryopreserved hepatocytes was analyzed.



	MK-571	
	-	+
AUC ((pmol/mg) × min)	5436	18743

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

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