

**BHCP-Pool-I Certified Cryopreserved Plateable Beagle Hepatocytes Pool for  
Induction assays  
Cell Specification**

Lot BH170718-4 – Pool of 3 donors

Batch Release: Nov 23, 2017

Species: Beagle  
Gender: male

Age Donor 1: 9 months  
Age Donor 2: 9 months  
Age Donor 3: 9 months

Cryopreservation:

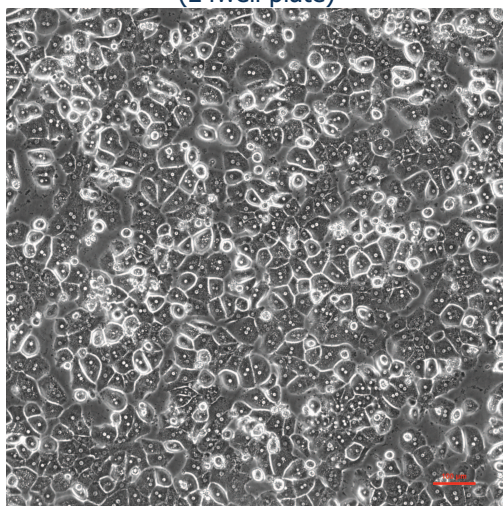
Date: July 18, 2017

Amount per vial:  $10.2 \times 10^6$  viable cells

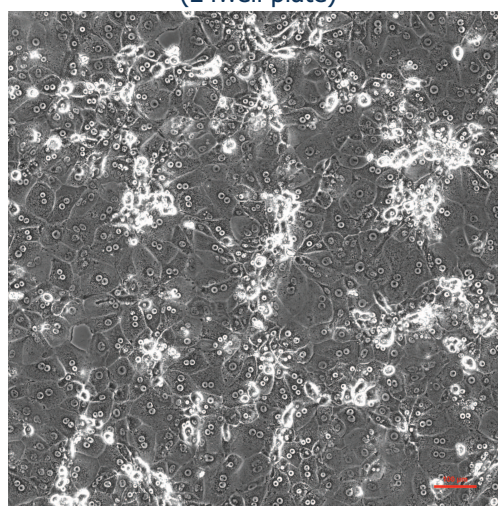
**Thawing:**

	Post-thaw viability (%)	Post-thaw yield per vial ( $\times 10^6$ )	Recovery (%)
<b>Pool (n=3)</b>	<b><math>89 \pm 1</math></b>	<b><math>6.5 \pm 0.1</math></b>	<b><math>64 \pm 1</math></b>
Donor 1 (n=3)	$82 \pm 7$	$6.1 \pm 0.1$	$60 \pm 1$
Donor 2 (n=2)	$85 \pm 7$	$5.4 \pm 0.5$	$53 \pm 4$
Donor 3 (n=2)	$89 \pm 1$	$6.4 \pm 0.7$	$62 \pm 7$
Mean Donor 1-3	$85 \pm 3$	$6.0 \pm 0.5$	$59 \pm 5$

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:

24well plate – 540,000 cells/well

96well plate – 90,000 cells/well

Culture in Human Hepatocyte Maintenance Medium (HHMM)

### Induction of CYP activity in plated hepatocytes:

n = 2, Mean  $\pm$  SD

CYP450 Protein (Beagle isoform)	<b>1A1/2</b>		
Inducer: Omeprazol	Basal activity	Induced activity	x-fold induction
<b>Pool</b>	7.1 $\pm$ 1.0	119.7 $\pm$ 10.2	17.0

Plated hepatocytes in 24well plates were treated with specific inducer or solvent control (0.2 % DMSO) from day 1 after thawing for 48 h. At day 3 after thawing, cell cultures were incubated with suitable substrate in culture medium HHMM for 30 min at 37 °C for determination of basal and induced CYP activity. Metabolite was quantified by LC-MS and normalized to protein content. Results are expressed in pmol/mg\*min.

### CYP activity in suspension cultures at day of thawing:

n=2, Mean  $\pm$  SD

CYP450 Protein (Beagle isoforms)	<b>1A1/2</b>	<b>2B11</b> (human 2B6)	<b>3A12/26</b> (human 3A4/5)
<b>Pool</b>	<b>135.6 <math>\pm</math> 6.7</b>	<b>74.4 <math>\pm</math> 4.8</b>	<b>9.4 <math>\pm</math> 0.4</b>
Donor 1	197.0 $\pm$ 45.0	62.9 $\pm$ 2.1	8.1 $\pm$ 0.4
Donor 2	115.5 $\pm$ 11.9	75.3 $\pm$ 2.3	6.2 $\pm$ 0.0
Donor 3	121.8 $\pm$ 5.3	72.4 $\pm$ 0.2	8.1 $\pm$ 0.2
Mean Donor 1-3	144.8 $\pm$ 45.4	70.2 $\pm$ 6.5	7.5 $\pm$ 1.1

Hepatocytes in suspension culture (0.5 \*10<sup>6</sup> cells 0.5 ml in HPM cryo) were incubated with specific substrates for 30 min at 37 °C for determination of CYP activities. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1000 rpm) in Eppendorf Thermomixer C. Metabolites were quantified by LC-MS and normalized to protein content. The substrates were applied as cocktail for simultaneous assessment of CYP 450 activity. Results are expressed in pmol/mg\*min.

### Viability in suspension cultures at day of thawing:

Time (h)	0	0.5	1.0	1.5	2.0	3.0	4.0	5.0
Viability (%)	87.0	79.3	81.8	83.5	79.7	88.0	71.8	69.6

Hepatocytes in suspension culture (0.5 \*10<sup>6</sup> cells in 0.5 ml HPM cryo) were incubated up to 5 h at 37 °C for viability determination. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1000 rpm) using Eppendorf Thermomixer C. Samples were taken at the indicated time points and the cell viability was determined.

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

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