

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

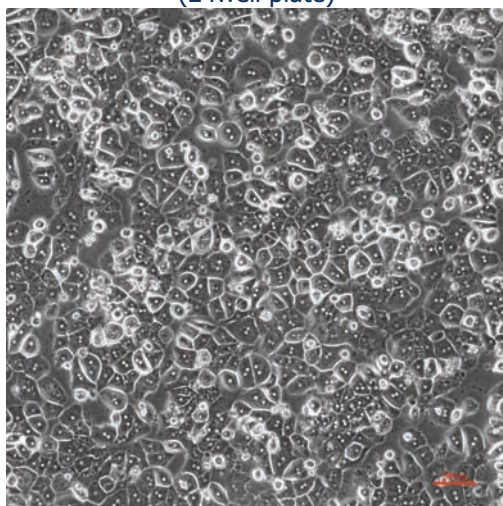
Species: Beagle Age Donor 1: 9 months
Gender: male Age Donor 2: 9 months
Age Donor 3: 9 months

Cryopreservation:
Date: July 18, 2017
Amount per vial: 10.2*10⁶ viable cells

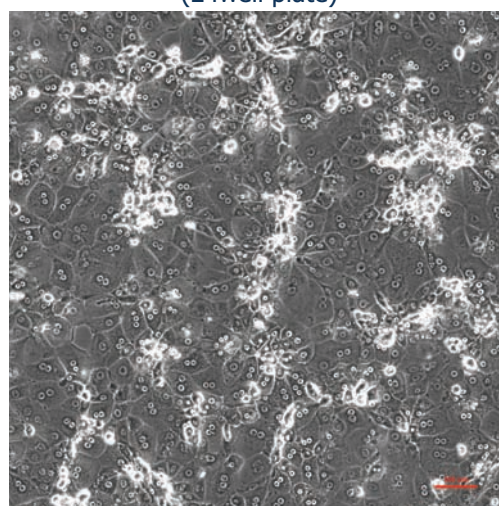
Thawing:

	Post-thaw viability (%)	Post-thaw yield per vial (*10 ⁶)	Recovery (%)
Pool (n=3)	89 ± 1	6.5 ± 0.1	64 ± 1
Donor 1 (n=3)	82 ± 7	6.1 ± 0.1	60 ± 1
Donor 2 (n=2)	85 ± 7	5.4 ± 0.5	53 ± 4
Donor 3 (n=2)	89 ± 1	6.4 ± 0.7	62 ± 7
Mean Donor 1-3	85 ± 3	6.0 ± 0.5	59 ± 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 ± SD

CYP450 Protein (Beagle isoform)	1A1/2		
Inducer: Omeprazol	Basal activity	Induced activity	x-fold induction
Pool	7.1 ± 1.0	119.7 ± 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 ± SD

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

Hepatocytes in suspension culture (0.5 *10⁶ 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 *10⁶ 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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