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Cell Culture Technology

CHCP-Pool-I Certified Cryopreserved Plateable Cynomolgus Hepatocytes Pool for Induction assays
Cell Specification

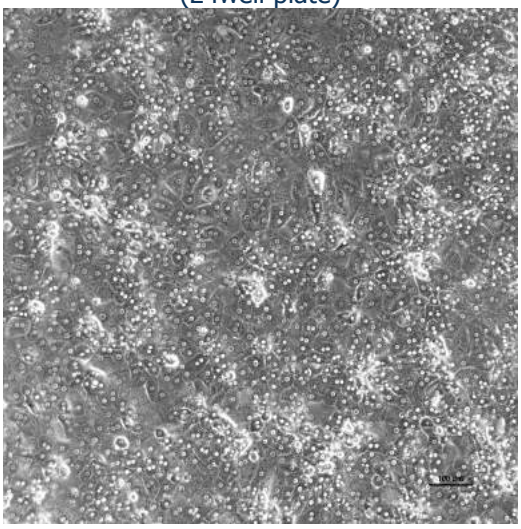
Lot CH170704-4 – Pool of 3 donors	Batch Release: Sept 19, 2017

Species: Macaca fascicularis Gender: male Age Donor 1: 4 years 10 months Age Donor 2: 5 years 8 months Age Donor 3: 4 years 9 months	Serology: All donors negative for Ebola, SRV, SIV, STLV-1, alpha-Herpes
Cryopreservation: Date: July 4, 2017 Amount per vial: $10.2*10^6$ viable cells	

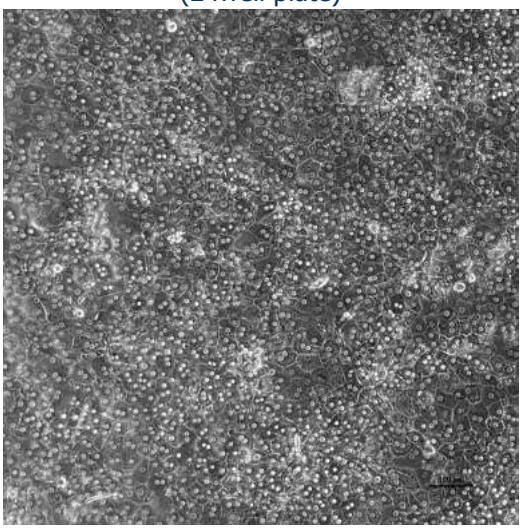
Thawing:

	Post-thaw viability (%)	Post-thaw yield per vial ($*10^6$)	Recovery (%)
Pool (n=3)	**96 ± 0**	**5.2 ± 0.6**	**51 ± 6**
Donor 1 (n=3)	96 ± 2	6.7 ± 0.8	66 ± 8
Donor 2 (n=2)	96 ± 3	7.3 ± 0.5	73 ± 5
Donor 3 (n=2)	95 ± 0	4.6 ± 0.3	47 ± 3
Mean Donor 1-3	96 ± 1	6.2 ± 1.4	62 ± 13

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 – 300,000 cells/well
96well plate – 70,000 cells/well
Culture in Human Hepatocyte Maintenance Medium (HHMM)

E-mail: info@primacyt.com, Tel.: +49 (0) 385-3993-600, Fax: +49 (0) 385-3993-602

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EROD activity in plated hepatocytes after thawing:

Mean ± SD, values in pmol/mg*min

24well plate			
CYP450 Protein (Cynomolgus isoform)	1A1/2		
Inducer: β-Naphthoflavone	Basal activity	Induced activity	x-fold induction
Pool	**7.1 ± 0.1**	**63.2 ± 15.1**	**8.9**
Donor 1	6.6 ± 0.3	40.4 ± 1.7	6.1
Donor 2	6.6 ± 2.3	32.1 ± 1.4	4.8
Donor 3	6.7 ± 0.5	57.8 ± 2.9	8.6
Mean Donor 1-3	6.6 ± 0.1	43.4 ± 13.1	6.5 ± 1.9

96well plate			
CYP450 Protein (Cynomolgus isoform)	1A1/2		
Inducer: β-Naphthoflavone	Basal activity	Induced activity	x-fold induction
Pool	**5.2 ± 0.8**	**47.3 ± 9.8**	**9.2**
Donor 1	4.7 ± 0.4	43.3 ± 9.7	9.3
Donor 2	5.8 ± 0.2	49.7 ± 4.5	8.6
Donor 3	6.7 ± 1.5	101.1 ± 11.8	15.0
Mean Donor 1-3	5.7 ± 1.0	64.7 ± 31.7	11.0 ± 3.5

Plated hepatocytes in 24well and 96well plates were treated with inducer β-Naphthoflavone or solvent control (0.5 % DMSO) from day 1 after thawing for 48 h. At day 3 after thawing, cell cultures were incubated with the substrate 7-Ethoxy-resorufin in Krebs-Henseleit buffer for 60 min at 37 °C for determination of basal and induced CYP activities. The metabolite Resorufin was quantified by fluorescence measurement and normalized to protein content. Results are expressed in pmol/mg*min.

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CYP activities in suspension cultures at day of thawing:

Mean ± SD, values in pmol/mg*min

CYP450 Protein (Cynomolgus isoforms)	**1A1/2**	**2B17** (human 2B6)	**3A8** (human 3A4/5)
Pool	**49.5 ± 0.7**	**227.8 ± 0.7**	**10.8 ± 0.4**
Donor 1	49.5 ± 6.7	221.2 ± 12.8	12.0 ± 1.7
Donor 2	59.1 ± 0.2	360.2 ± 25.2	16.7 ± 0.3
Donor 3	41.6 ± 0.3	85.7 ± 1.3	9.1 ± 0.1
Mean Donor 1-3	50.0 ± 8.7	222.4 ± 137.2	12.6 ± 3.9

Hepatocytes in suspension culture (0.5 *10^6 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.

Substrates used for determination of CYP activities:

CYP450 Protein (Cynomolgus isoform)	**1A1/2**	**2B17**	**3A8**
Substrate	Phenacetin	Bupropion	Midazolam
Metabolite	Acetaminophen	Hydroxybupropion	1-OH-Midazolam

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

Issued by: A. Ullrich Checked by: J. Jia

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