

**CHCP-I-3D Cynomolgus Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture**

**Cell Specification – Certificate of Analysis (CoA)**

Lot CH220608

Batch Release: October 21, 2022

**Donor data**

Species: *Macaca fascicularis*

Gender: female

Age: 5 years 8 months

The animals from which the samples were taken were examined and found healthy. The stock is under regular veterinarian surveillance. Bacteriological and parasitological controls are performed. Causes of death are determined regularly. All animals used come from veterinary controlled breeding in Mauritius. Mauritius animals are not tested for viruses like SIV, SRV, STLV+BV, as they are considered free of them due to their origin.

**Cryopreservation and Thawing**

**Cryopreservation:**

Date: June 8, 2022

Amount per vial:  $10.0 \times 10^6$  cells

**Thawing: n=2**

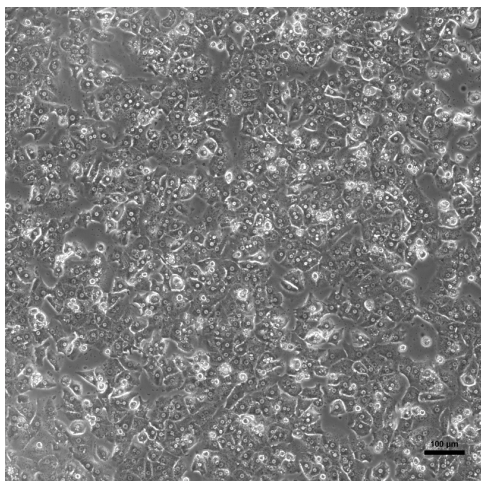
Post-thaw viability:  $93.3 \pm 0.8 \%$

Post-thaw yield per vial:  $5.5 \pm 0.5 \times 10^6$  cells

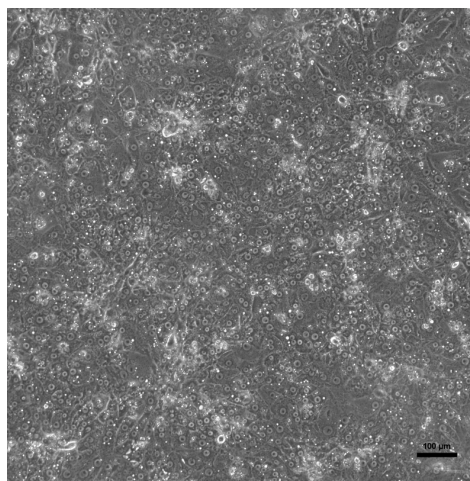
Recovery: 54 %

**2D culture**

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 – 500,000 cells/well // 96well plate – 90,000 cells/well

Culture in Human Hepatocyte Maintenance Medium (HHMM).

CYP P450 activity in 2D culture after thawing:

pmol/(mg × min)

X-fold induction

Ethoxyresorufin-O-deethylation:

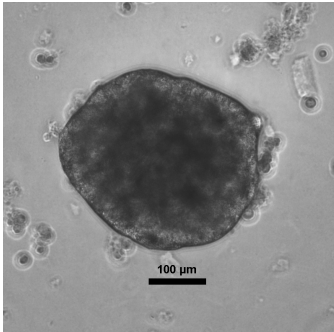
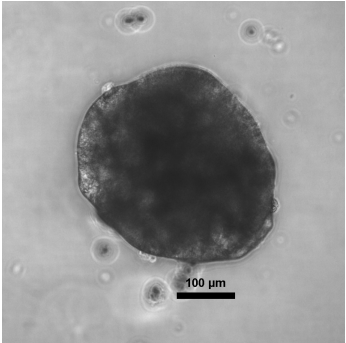
24well:  $18.4 \pm 0.6$

2.8

Induction with 25 µM β-Naphthoflavone

96well:  $29.4 \pm 4.8$

3.3

3D culture		
Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well		
day 7	day 9	
		
CYP P450 activity in 3D culture after thawing: Ethoxyresorufin-O-deethylation: Induction with 3 µM β-Naphthoflavone	pmol/(mg × min)  7.8 ± 2.3	X-fold induction  2.6

Suspension culture						
Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10 <sup>6</sup> cells in 0.5 ml HPM-Cryo):						
Time (h)	0	1	2	3	4	5
Viability (%)	92,5	90.6	87.7	89.2	87.0	86.2

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

**Store at -150 °C or in the vapour phase of LN<sub>2</sub>**

This product should be considered as potential biohazard. Only intended for *in vitro* use.

Issued by: A. Ullrich	Verified by: T. Krimmling
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