

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

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

Lot CH220929-1B

Batch Release: March 27, 2023

**Donor data**

Species: *Macaca fascicularis*

Gender: male

Age: 3 years 7 months

Serology: negative for Herpes B virus, SRV, SIV, STLV-1, Filovirus (Ebola-like)

**Cryopreservation and Thawing**

**Cryopreservation:**

Date: Sept 29, 2022

Amount per vial:  $5.0 \times 10^6$  cells

**Thawing:** n=1

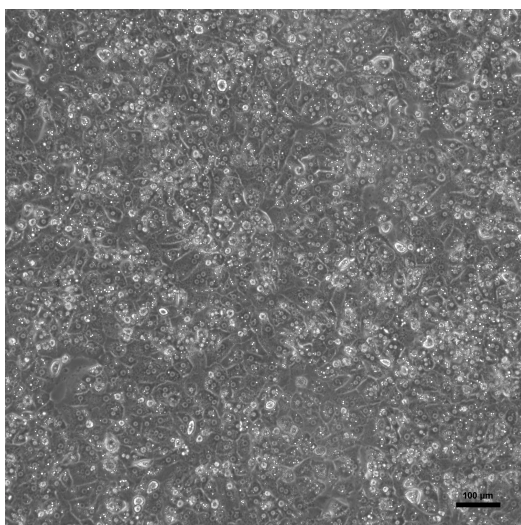
Post-thaw viability: 93.3 %

Post-thaw yield per vial:  $3.1 \times 10^6$  cells

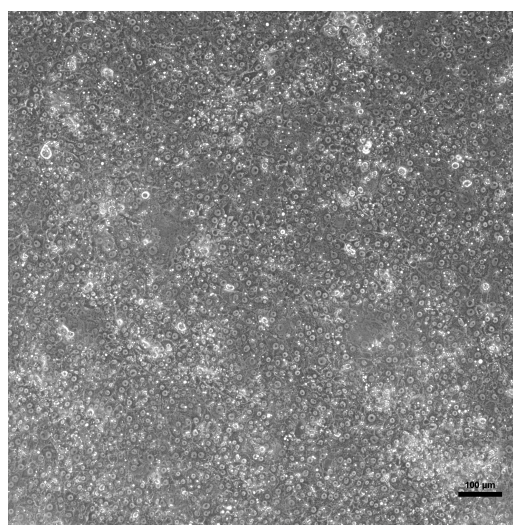
Recovery: 61 %

**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 – 400,000 cells/well // 96well plate – 70,000 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:  $68.2 \pm 10.7$

16.4

Induction with 25 µM β-Naphthoflavone

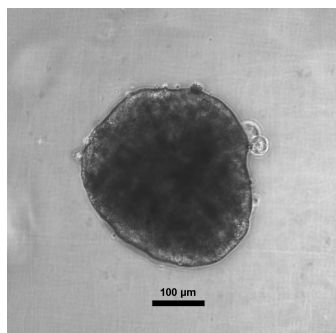
96well:  $149.5 \pm 55.3$

24.6

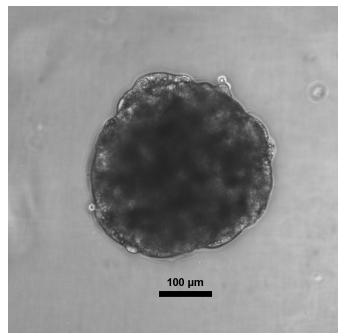
### 3D culture

Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well

day 4



day 9



### Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with  $0.5 \times 10^6$  cells in 0.5 ml HPM-Cryo):

Time (h)	0	1	2	3	4
Viability (%)	93.3	71.7	71.0	64.3	63.1

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: K. Damrau