

**CHCP-I-T Cynomolgus Hepatocytes Cryopreserved Plateable for Induction and Transporter Assays**

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

Lot CH130515-1

Batch Release: July 9, 2013, update: April 20, 2023

**Donor data**

Species: *Macaca fascicularis*

Gender: female

Age: 5 years 2 months

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

**Cryopreservation and Thawing**

**Cryopreservation:**

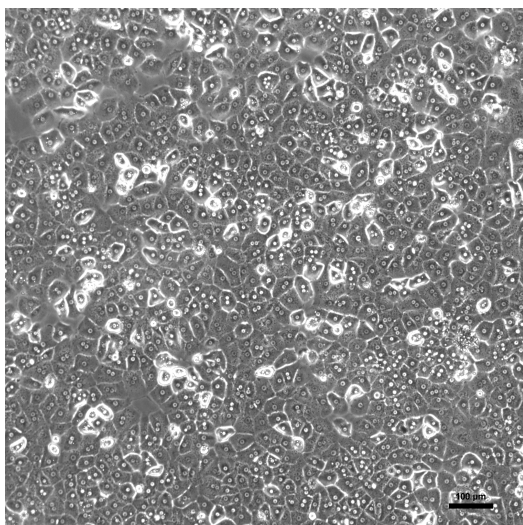
Date: May 15, 2013  
Amount per vial:  $10.0 \times 10^6$  cells

**Thawing:** n=5

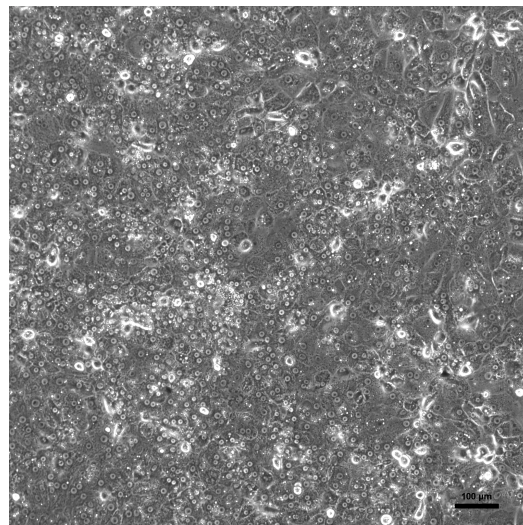
Post-thaw viability:  $92.0 \pm 4.3$  %  
Post-thaw yield per vial:  $7.2 \pm 1.3 \times 10^6$  cells  
Recovery: 72 %

**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 – 300,000 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:  $155.5 \pm 4.4$

11.1

Induction with 25 μM β-Naphthoflavone

96well: 273.9

14.0

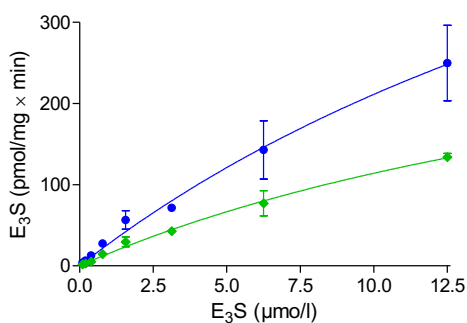
### Suspension culture

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    |
| Viability (%) | 93.2 | 76.3 | 89.6 |

### Transporter assays

Uptake transporters: uptake of Estrone 3-sulfate ( $E_3S$ ) with (green) or without (blue) competitive inhibitor Bromosulphophthalein (BSP, 100  $\mu M$ ) in cryopreserved hepatocytes after 2 min incubation.



|       | $K_m$<br>( $\mu\text{mol/l}$ ) | $V_{max}$<br>( $\text{pmol/mg} \times \text{min}$ ) |
|-------|--------------------------------|---|
| - BSP | $29.3 \pm 14.3$                | $829 \pm 305$                                       |
| + BSP | $24.8 \pm 6.3$                 | $397 \pm 73$  |

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