

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

Cell Specification – Certificate of Analysis (CoA)

Lot CH130806

Batch Release: November 11, 2021; update April 18, 2023

Donor data

Species: *Macaca fascicularis*

Gender: male

Age: 4 years 7 months

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

Cryopreservation and Thawing

Cryopreservation:

Date: August 6, 2013

Amount per vial: 10.1×10^6 cells

Thawing: n=4

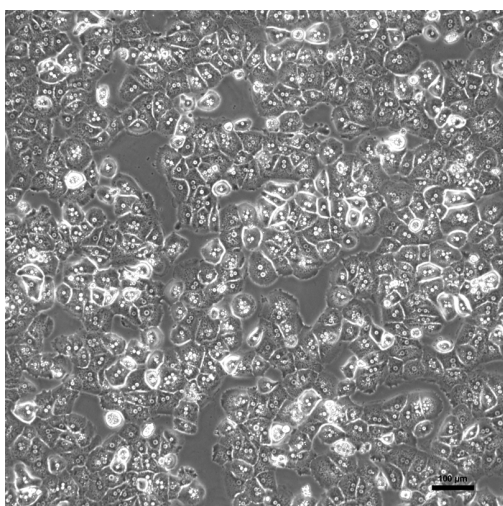
Post-thaw viability: 84.1 ± 5.1 %

Post-thaw yield per vial: $6.7 \pm 0.8 \times 10^6$ cells

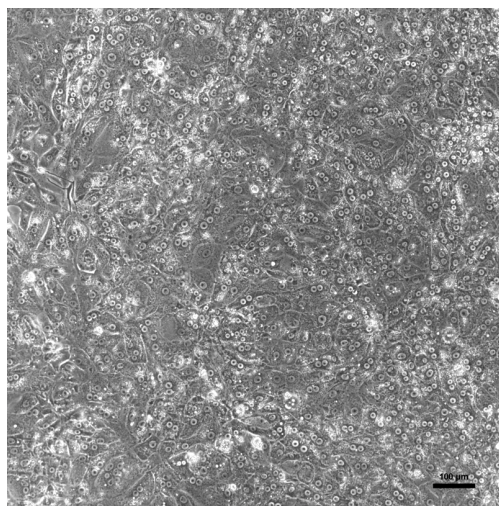
Recovery: 66 %

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

Culture in Human Hepatocyte Maintenance Medium (HHMM).

CYP P450 activity in 2D culture after thawing:

Ethoxyresorufin-O-deethylation:

Induction with 25 μ M β -Naphthoflavone

pmol/(mg $\times$ min)

24well: 125.8 ± 7.3

X-fold induction

6.0

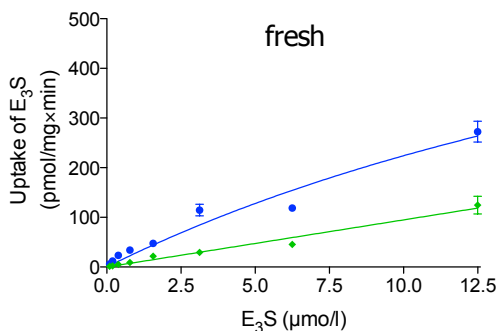
Suspension culture

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

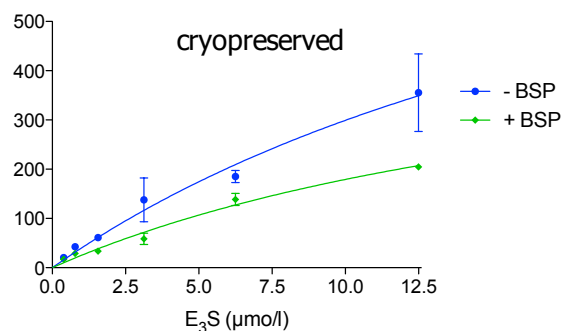
Time (h)	0	1	2	3
Viability (%)	87.6	82.5	80.7	76.4

Transporter assays

Uptake Transporters: uptake of Estrone 3-sulfate (E_3S) with (green) or without (blue) competitive inhibitor Bromosulphophthalein (BSP, 100 μM) in fresh and cryopreserved hepatocytes after 2 min incubation.



	- BSP	+ BSP
K_m ($\mu mol/l$)	30.2 ± 14.9	-
V_{max} (pmol/mg $\times$ min)	902 ± 339	-



	- BSP	+ BSP
K_m ($\mu mol/l$)	25.1 ± 14.5	21.0 ± 5.8
V_{max} (pmol/mg $\times$ min)	1050 ± 441	556 ± 107

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 LN₂

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

Issued by: A. Ullrich

Verified by: K. Damrau