

BHCP-I-3D Beagle Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture
Cell Specification – Certificate of Analysis (CoA)

Lot BH180829

Batch Release: Nov 23, 2017
Update: Oct 29, 2024

Donor data

Species: Beagle

Gender: male
Age: 7.5 months

Cryopreservation and Thawing

Cryopreservation:

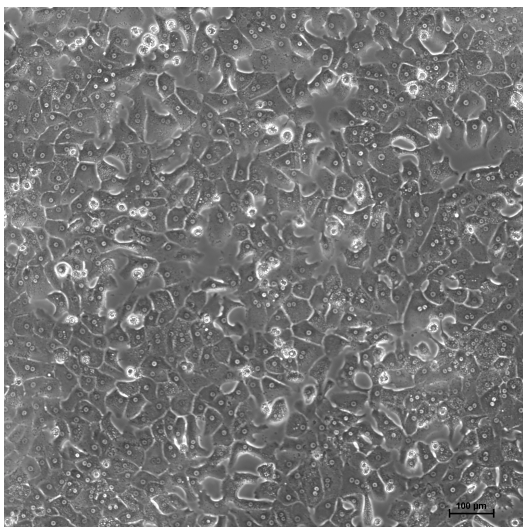
Date: Aug 29, 2018
Amount per vial: 10.5×10^6 cells

Thawing: n=6

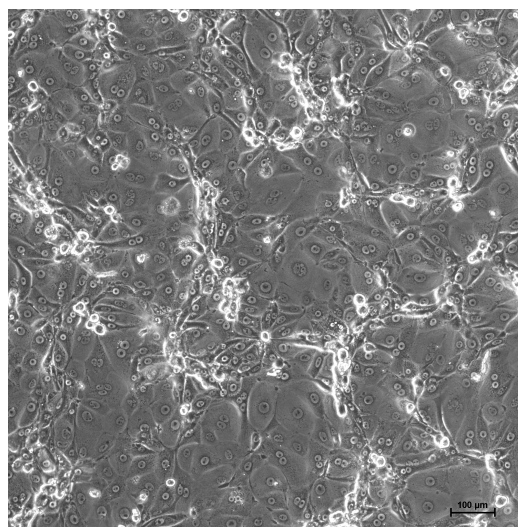
Post-thaw viability: $90.8 \pm 1.5 \%$
Post-thaw yield per vial: $6.1 \pm 1.6 \times 10^6$ cells
Recovery: $58 \pm 15 \%$

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:

12well plate – 800,000 cells/well // 24well plate – 400,000 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: 107.9 ± 5.8

13.2

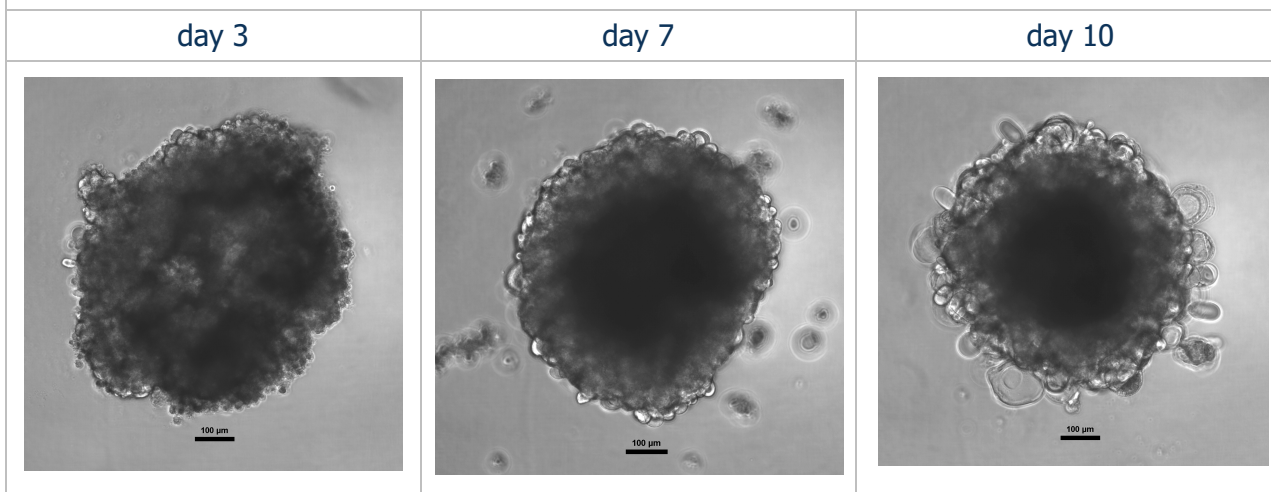
Induction with 25 µM β-Naphthoflavone

96well: 198.9 ± 16.7

18.0

3D culture

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



Suspension culture

Determination of CYP activities in suspension (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5×10^6 cells in 0.5 ml HPM Cryo medium with 5 % FCS):

Assay	Enzyme activities (pmol/min*mg protein)
	mean \pm SD
Phenacetin-O-deethylase	70.3 \pm 1.4
Bupropion-hydroxylase	21.7 \pm 0.6
Midazolam 1'-hydroxylase	47.8 \pm 3.8
UDP-Glucuronosyltransferase	180.1 \pm 3.8
Sulfotransferase	44.5 \pm 1.2

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 LN₂

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

Issued by: A. Ullrich

Verified by: K. Damrau