

BHCP-I-3D Beagle Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture

Cell Specification – Certificate of Analysis (CoA)

Lot BH181205

Batch Release: July 24, 2020

Update: Oct 28, 2024

Donor data

Gender: male Species: Beagle Age: 11 months

Cryopreservation and Thawing

Cryopreservation:

Date: Dec 5, 2018

10.0 x 10⁶ cells Amount per vial:

Thawing: n=2

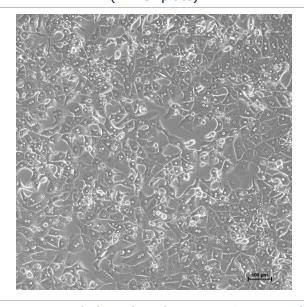
Post-thaw viability: 88.4 \pm 1.2 %

Post-thaw yield per vial: $6.0 \pm 0.2 \times 10^6$ cells

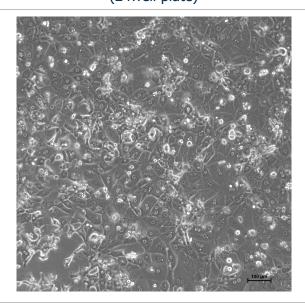
Recovery: $60 \pm 2 \%$

2D culture

Phase contrast on day 2 after thawing (24well plate)



Phase contrast on day 3 after thawing (24well plate)



Recommended seeding density on Corning collagen-coated plates:

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:

Ethoxyresorufin-O-deethylation:

Induction with 25 μM β-Naphthoflavone

 $pmol/(mg \times min)$ 24well: 94.8 ± 8.8

X-fold induction

11.1 5.4

96well: 410.4 ± 225.1

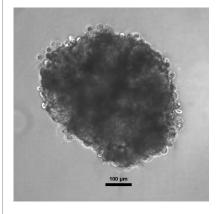
Page 1 of 2

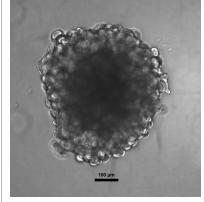


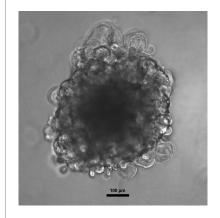
3D culture

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

day 3 day 7 day 10







Suspension culture

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

Time (h)	0	1	2
Viability (%)	89.6	84.8	83.3

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