

RHCP-I-3D Rat Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture

Cell Specification – Certificate of Analysis (CoA)

Lot RH220323

Batch Release: July 10, 2024

Donor data

Species: *Rattus norvegicus* forma domestica
Strain: Wistar:Han IGS rat // Crl:Wi(Han)

Gender: female
Age: approx. 1 year

Cryopreservation and Thawing

Cryopreservation:

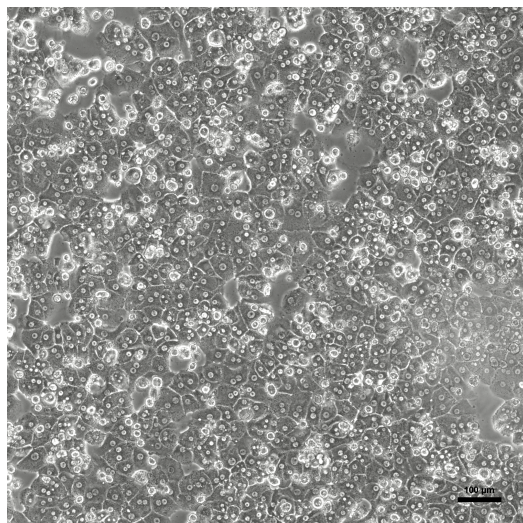
Date: March 23, 2022
Amount per vial: 10×10^6 cells

Thawing: n=2

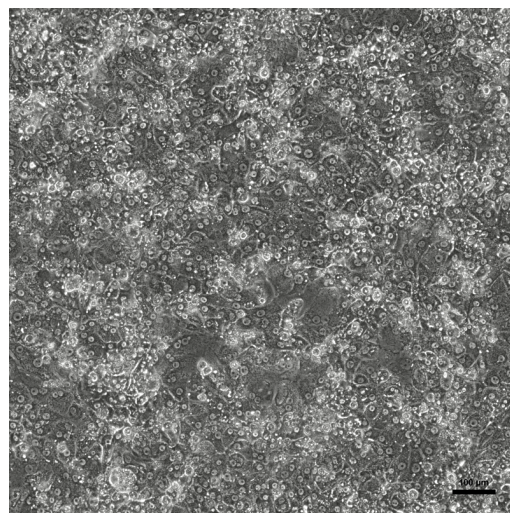
Post-thaw viability: 90.7 ± 0.5 %
Post-thaw yield per vial: $6.0 \pm 0.3 \times 10^6$ cells
Recovery: 60.4 ± 3.8 %

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 – 200,000 cells/well // 96well plate – 30,000 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:

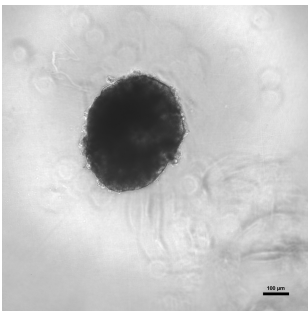
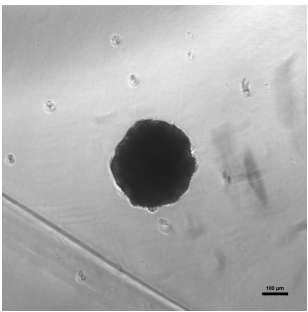
24well: 12.0 ± 1.0

5.3

Induction with 25 μM β-Naphthoflavone

96well: 57.9 ± 14.8

9.2

3D culture		
Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well		
day 7	day 14	
		
scale bar 100 µm	scale bar 100 µm	
CYP P450 activity in 3D culture after thawing: Ethoxyresorufin-O-deethylation: Induction with 3 µM β-Naphthoflavone	pmol/ (mg × min) 96well: 13.1 ± 1.1	X-fold induction 8.0

Suspension culture						
Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10 ⁶ cells in 0.5 ml HM-Cryo):						
Time (h)	0	1	2	3	4	5
Viability (%)	90.7	75.0	75.5	73.4	74.5	72.5
<p>Note: Yield, viability, recovery and activity assays were performed at PRIMACYT using PRIMACYT's manual for thawing, plating and culture of primary cryopreserved hepatocytes.</p> <p>Store at -150 °C or in the vapour phase of LN₂</p> <p>This product should be considered as potential biohazard. Only intended for <i>in vitro</i> use.</p>						

Detailed animal information and husbandry conditions			
Species	Rat (<i>Rattus norvegicus</i> forma domestica)		
Strain	Wistar:Han IGS rat // Crl:Wi(Han), IGS (International Genetic Standard)		
Vendor	Charles River Germany GmbH & Co. KG, Sulzbach, FRG		
Food	Altromin Maintenance diet for rats/mice (ad libitum), Altromin, Lage, FRG		
Water	ad libitum		
Light/Dark cycle	regulated day / night cycle using artificial and natural light, 10-14 hours light daily		
Temperature	20-24 °C		
Humidity	40-70 %		
Bedding	Hugro hemp bedding, Saerbeck, FRG		
Cage	Tecniplast Eurostandard Type III and Type IV incl. behavioural enrichments for the animals		
<p>Animals were housed under veterinary control and allowed to acclimate ≥ 7 days before use. Animal housing permits according to §11 Abs. 1 TSchG, dated May 15, 2008 and March 20, 2017 and March 22, 2022 under supervision of Veterinary Office of Landkreis Ludwigslust/Parchim, FRG. Hepatocytes were obtained from a non-infectious, non-contagious, healthy animal. The animal does not originate from a facility conducting work or research with animal pathogens.</p>			
<table border="1"> <tr> <td>Issued by: A. Alkufairi</td><td>Verified by: K. Damrau</td></tr> </table>		Issued by: A. Alkufairi	Verified by: K. Damrau
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