

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

Cell Specification – Certificate of Analysis (CoA)

Lot RH210310

Batch Release: November 11, 2021; updated July 10, 2022

Donor data

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

Gender: female
Age: approx. 5 months

Cryopreservation and Thawing

Cryopreservation:

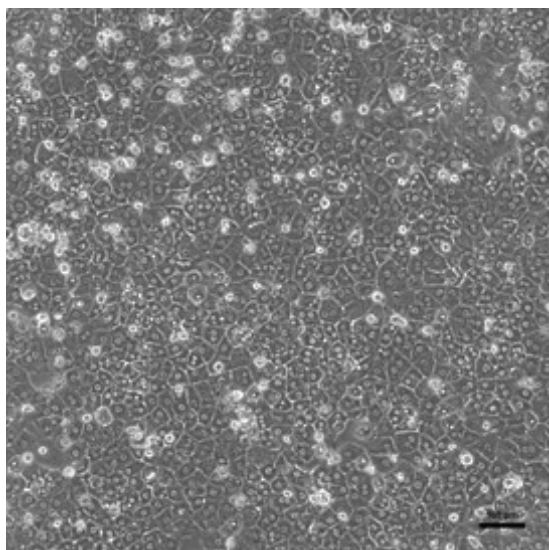
Date: Mar 10, 2021
Amount per vial: 10.1×10^6 cells

Thawing: n=1

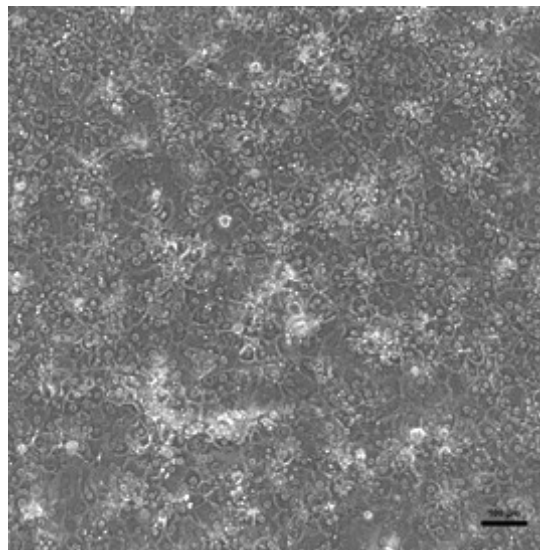
Post-thaw viability: 90.0 %
Post-thaw yield per vial: 7.0×10^6 cells
Recovery: 70 %

2D culture

Phase contrast on day 1 after thawing
(24well plate)

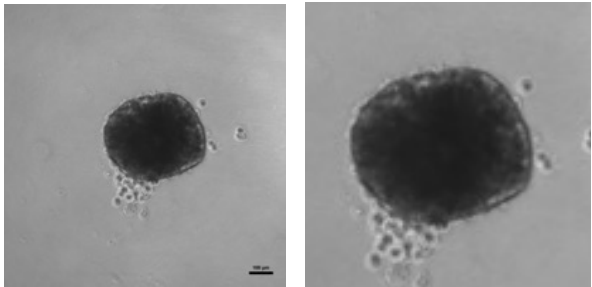
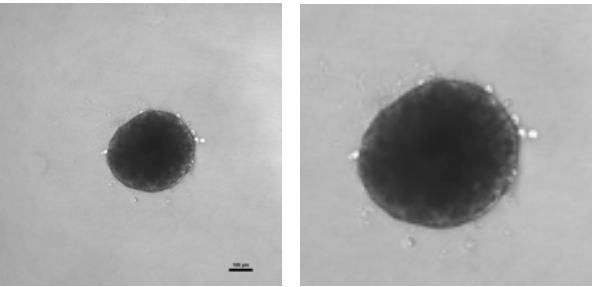


Phase contrast on day 3 after thawing
(24well plate)



Recommended seeding density on collagen-coated plates:
24well plate – 300,000 cells/well // 96well plate – 50,000 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: 135.3 ± 1.1	17.5
Induction with 25 μ M β -Naphthoflavone	96well: 472.6 ± 89.4	32.5

3D culture		
Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well		
day 4		day 7
 <p>scale bar 100 µm</p>		 <p>scale bar 100 µm</p>
CYP P450 activity in 3D culture after thawing: Ethoxyresorufin-O-deethylation: Induction with 3 µM β-Naphthoflavone	pmol/(mg × min) 96well: 11.3 ± 1.8	X-fold induction 2.6

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	0.5	1	1.5	2	3	4	5
Viability (%)	90.0	67.2	75.8	67.3	73.6	74.3	69.5	78.0
<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	07:00-19:00 light / 19:00-07:00 dark (12 hours light/dark)
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 permit according to §11 Abs. 1 TSchG, dated March 20, 2017 under supervision of Veterinary Office of Landkreis Ludwigslust/Parchim, FRG. Hepatocytes were obtained from a non-infectious, non-contagious, healthy animal.</p>	
Issued by: A. Ullrich	Verified by: Dieter Runge