

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

**Cell Specification – Certificate of Analysis (CoA)**

Lot RH210204

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

**Donor data**

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

Gender: female  
Age: approx. 3.5 months

**Cryopreservation and Thawing**

**Cryopreservation:**

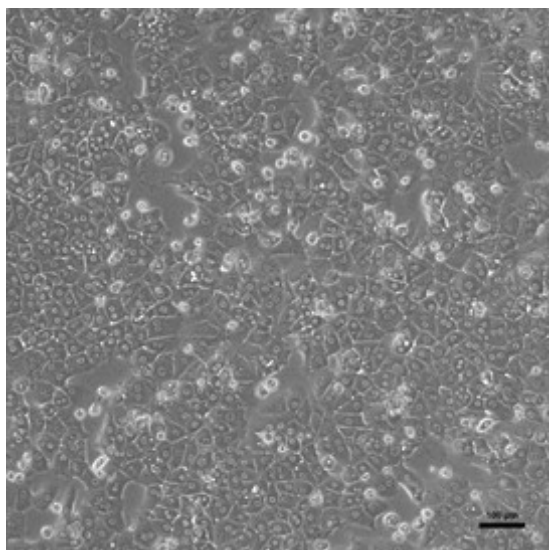
Date: Feb 04, 2021  
Amount per vial:  $10.0 \times 10^6$  cells

**Thawing:** n=4

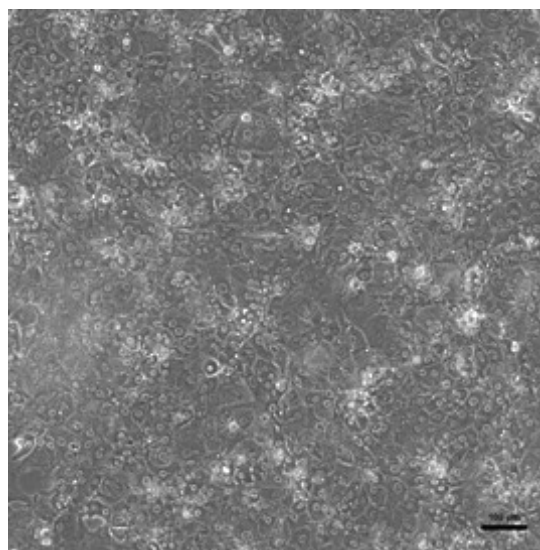
Post-thaw viability:  $73.7 \pm 7.3$  %  
Post-thaw yield per vial:  $6.8. \pm 0.9 \times 10^6$  cells  
Recovery: 68 %

**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:

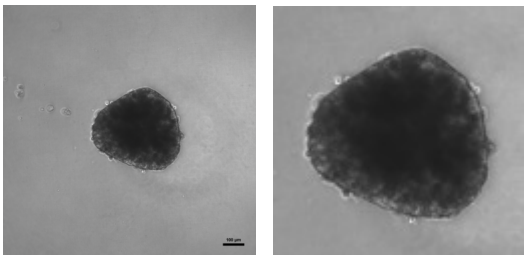
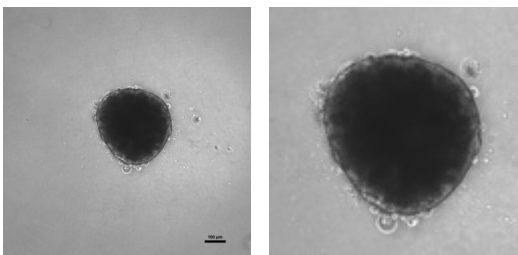
24well:  $115.0 \pm 4.4$

18.9

Induction with 25 μM β-Naphthoflavone

96well:  $456.0 \pm 125.9$

25.6

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: 13.6 ± 3.6	X-fold induction  4.2

Suspension culture							
Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10 <sup>6</sup> cells in 0.5 ml HM-Cryo):							
Time (h)	0	0.5	1	1.5	2	3	4
Viability (%)	73.7	68.4	63.6	67.3	63.2	68.0	61.1
<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><b>Store at -150 °C or in the vapour phase of LN<sub>2</sub></b></p> <p>This product should be considered as potential biohazard. Only intended for <i>in vitro</i> use.</p>							

<b>Detailed animal information and husbandry conditions</b>	
Species	Rat ( <i>Rattus norvegicus</i> forma domestica)
Strain	Wistar:Han IGS rat // CrI: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 <math>\geq 7</math> days before use. Animal housing permits according to §11 Abs. 1 TSchG, dated 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>	
Issued by: A. Ullrich	Verified by: Dieter Runge