

**RHCP-I Cryopreserved Rat Hepatocytes Plateable for Induction assays**  
**Cell Specification – Certificate of Analysis (CoA)**

Lot RH191007

Batch Release: August 01, 2023

**Donor data**

Species: *Rattus norvegicus* forma domestica  
CD IGS rat (Sprague Dawley) // CrI:CD(SD)

Gender: male  
Age: approx. 2 months

**Cryopreservation and Thawing**

**Cryopreservation:**

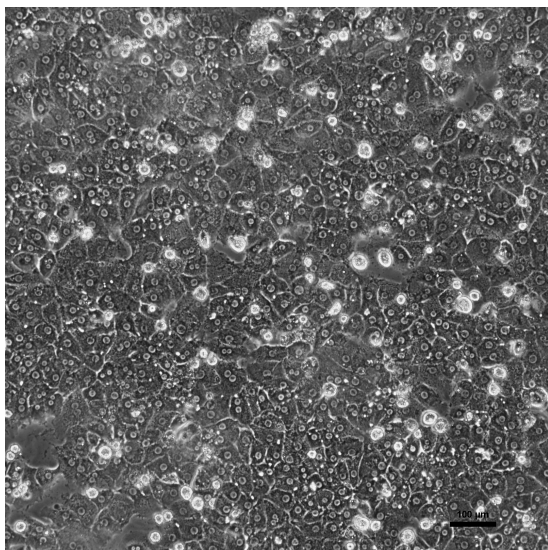
Date: Oct 07, 2019  
Amount per vial:  $10.0 \times 10^6$  cells

**Thawing:** n=2

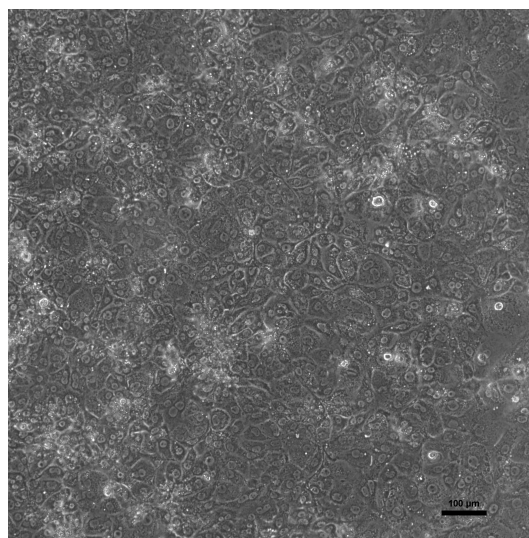
Post-thaw viability:  $86.1 \pm 1.3$  %  
Post-thaw yield per vial:  $5.0 \pm 0.5 \times 10^6$  cells  
Recovery:  $50.0 \pm 5.2$  %

**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 – 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:  $89.5 \pm 8.2$

23.9

Induction with 25 µM β-Naphthoflavone

96well:  $110.7 \pm 11.0$

9.0

### Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with  $0.5 \times 10^6$  cells in 0.5 ml HM-Cryo):

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	86.1 ±1.3	65.2 ±0.3	67.2 ±4.2	57.8 ±2.0	54.6 ±3.5	55.4	50.0	50.0

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 LN<sub>2</sub>

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

### Detailed animal information and husbandry conditions

Species	Rat ( <i>Rattus norvegicus</i> forma domestica)
Strain	CD IGS rat (Sprague Dawley) // CrI:CD(SD), 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

Animals were housed under veterinary control and allowed to acclimate  $\geq 7$  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.

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