

RHCP-I Rat Hepatocytes Cryopreserved Plateable for Induction assays

Cell Specification – Certificate of Analysis (CoA)

Lot RH220314

Batch Release: August 10, 2023

Donor data

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

Gender: female
Age: approx. 12 months

Cryopreservation and Thawing

Cryopreservation:

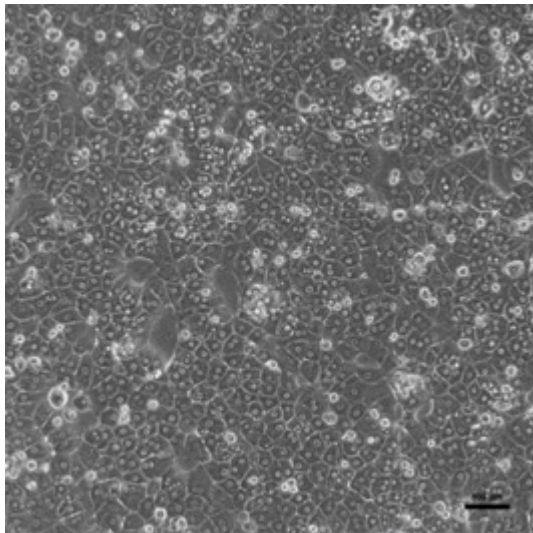
Date: March 14, 2022
Amount per vial: 10.067×10^6 cells

Thawing: n=2

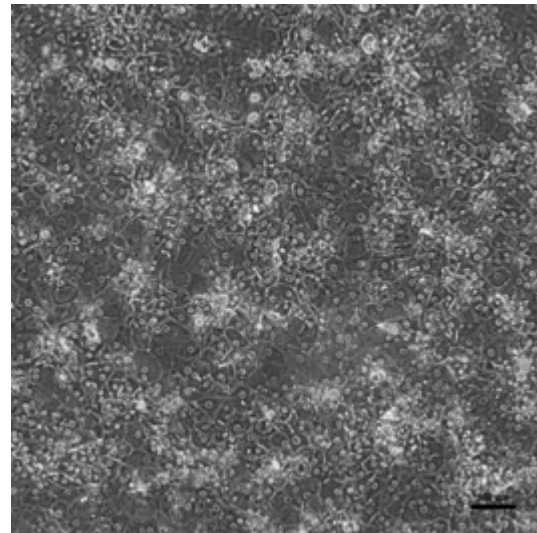
Post-thaw viability: 94.7 ± 1.4 %
Post-thaw yield per vial: $6.3 \pm 0.2 \times 10^6$ cells
Recovery: 63.2 ± 2.1 %

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: 35.4 ± 1.5	12.7
Induction with 25 μ M β -Naphthoflavone	96well: 57.1 ± 8.3	11.8

Suspension culture

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

Time (h)	0	1	2	3	4	5
Viability (%)	94.7	82.8	84.1	77.9	77.7	81.9

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.

Detailed animal information and husbandry conditions

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	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 ≥ 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.

Issued by: A. Alkufairi

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