

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

Lot RH200226-1 (Pool of 5)

Batch Release: December 15, 2025

Donor data

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

Gender: 5 females
Age: approx. 5 months

Cryopreservation and Thawing

Cryopreservation:

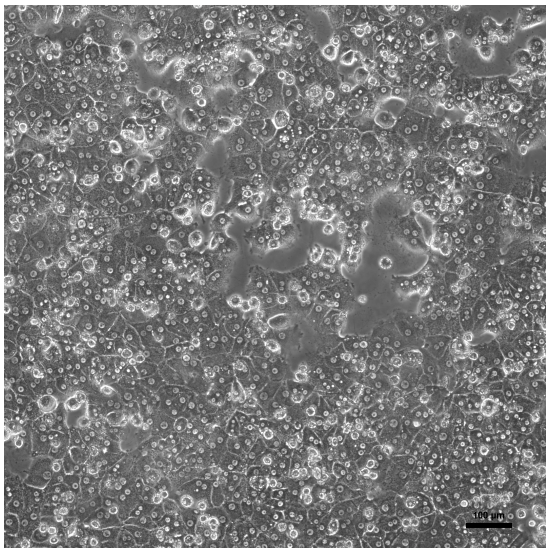
Date: Feb 26, 2020
Amount per vial: 12.0×10^6 cells

Thawing: n=3

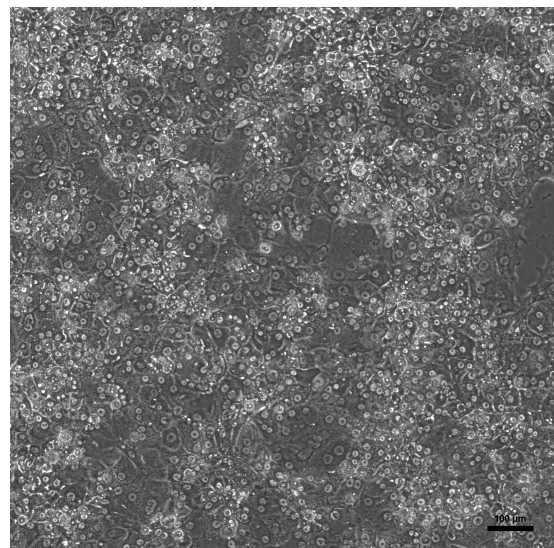
Post-thaw viability: 81.6 ± 6.2 %
Post-thaw yield per vial: $7.7 \pm 0.4 \times 10^6$ cells
Recovery: 64 %

2D culture

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



Phase contrast on day 2 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).

Note: Gently shake the plate (N/S-E/W) every 30 minutes for 2 hours after plating (only 24well plate and bigger wells). This step has a positive effect on the uniform plating.

CYP P450 activity in 2D culture after thawing: Ethoxyresorufin-O-deethylation: Induction with 25 µM β-Naphthoflavone	n = 2 pmol/ (mg × min) 24well: 77.2 ± 15.7 96well: 190.6 ± 80.8	n = 2 X-fold induction 20.8 23.0
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Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 °C with 0.5 x 10⁶ cells in 0.5 ml HM-Cryo), n = 2:

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	81.4	68.3	71.9	66.2	60.4	57.9	63.1	61.1

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 (Rattus norvegicus forma domestica)
Strain	CD IGS rat (Sprague Dawley) // CrI:CD(SD), 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, March 22, 2022 and November 18, 2024 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: M. Reu

Verified by: J. Schuldt