

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

Lot RH240226

Batch Release: June 25, 2024

**Donor data**

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

Gender: male  
Age: approx. 1.5 months

**Cryopreservation and Thawing**

**Cryopreservation:**

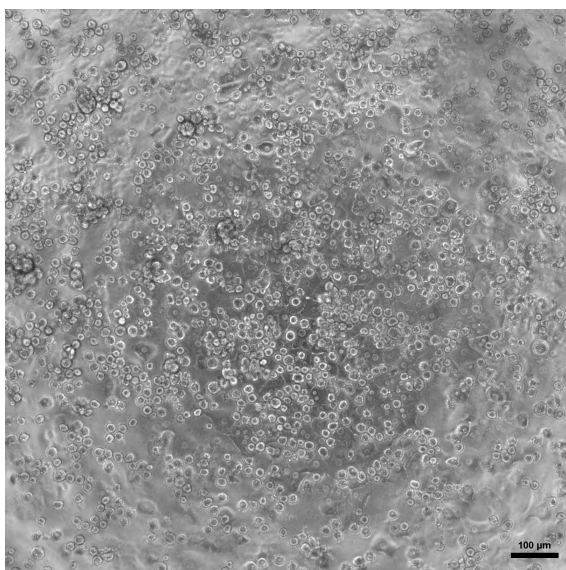
Date: Feb 26, 2024  
Amount per vial:  $10.3 \times 10^6$  cells

**Thawing:** n=1

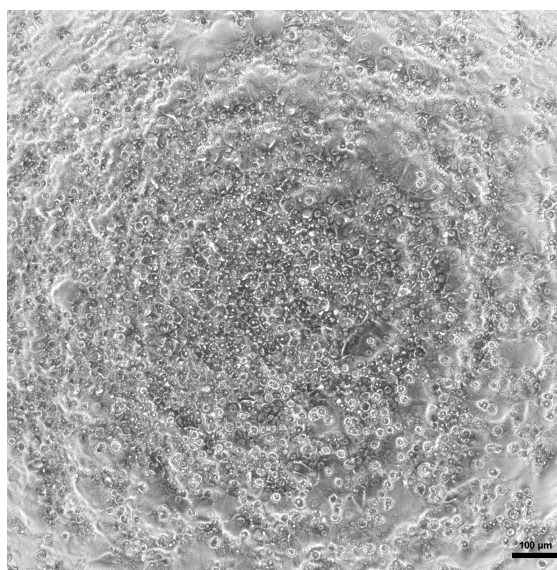
Post-thaw viability: 82.4 %  
Post-thaw yield per vial:  $5.7 \times 10^6$  cells  
Recovery: 55.7 %

**2D culture**

Phase contrast on day 1 after thawing  
(96well plate)



Phase contrast on day 3 after thawing  
(96well 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: $76.2 \pm 4.1$ | 26.1             |
| Induction with 25 µM β-Naphthoflavone          | 96well: $82.2 \pm 7.6$ | 30.8             |

### 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    |
| Viability (%) | 82.4 | 71.4 | 55.6 | 52.5 | 56.3 |

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           | Wistar:Han IGS rat // CrI:Wi(Han), IGS (International Genetic Standard)                    |
| Vendor           | Janvier Labs, Saint Berthevin Cedex, France  |
| 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 May 15, 2008 and 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. Sattler

Verified by: K. Damrau