

**MHCP-I-3D Mouse Hepatocytes Cryopreserved Plateable for Induction assays and for 3D culture**

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

Lot MH230403

Batch Release: Dec. 15, 2023

**Donor data**

Species: Mouse (*Mus musculus*)  
Strain: CD-1 SWISS

Gender: 7 females  
Age: approx. 2 months

**Cryopreservation and Thawing**

**Cryopreservation:**

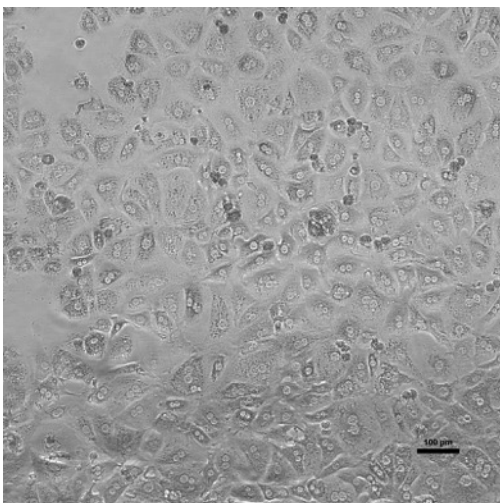
Date: Apr 03, 2023  
Amount per vial:  $7.1 \times 10^6$  cells

**Thawing:** n=2

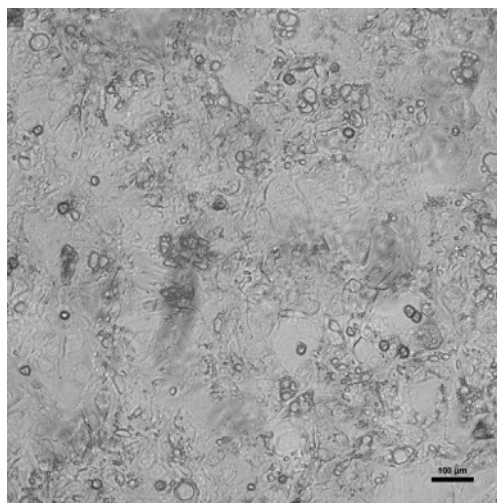
Post-thaw viability:  $91.1 \pm 3.1$  %  
Post-thaw yield per vial:  $3.7 \pm 0.5 \times 10^6$  cells  
Recovery:  $52.4 \pm 7.5$  %

**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 – 120,000 cells/well // 96well plate – 25,000 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:  $19.22 \pm 3.05$

9.5

Induction with 25 µM β-Naphthoflavone

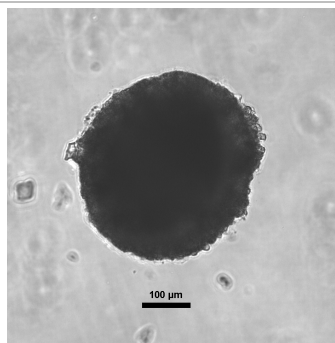
96well:  $39.09 \pm 8.9$

8.2

### 3D culture

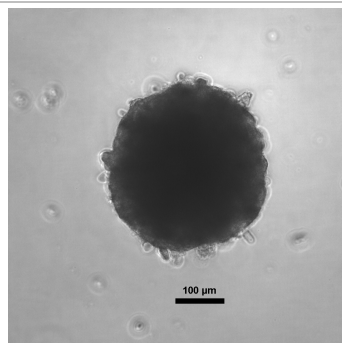
Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well

day 4



scale bar 100 µm

day 8



scale bar 100 µm

### 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 HPM-Cryo):

Time (h)	0	0.5	1	1.5	2	3
Viability (%)	91.1	69.3	72.2	73.8	60.3	63.0

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	Mouse (Mus musculus)
Strain	CD-1 SWISS, 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
<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. Sattler	Verified by: T. Krimmling