

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

Cell Specification – Certificate of Analysis (CoA)

Lot MH210907

Batch Release: May 08, 2024

Donor data

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

Gender: male
Age: approx. 2 months

Cryopreservation and Thawing

Cryopreservation:

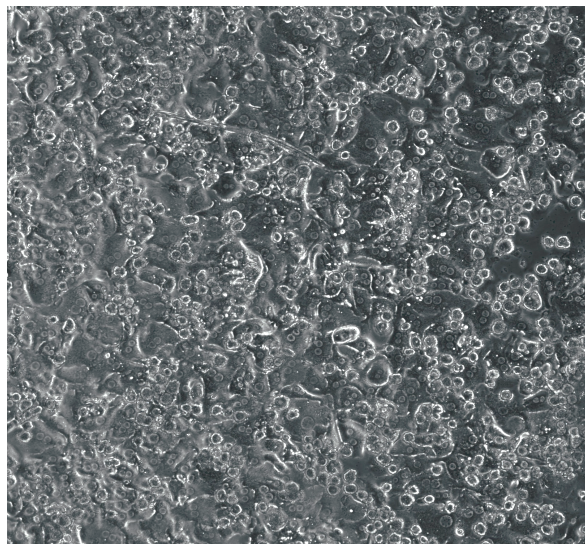
Date: Sep. 07, 2021
Amount per vial: 6.19×10^6 cells

Thawing: n=2

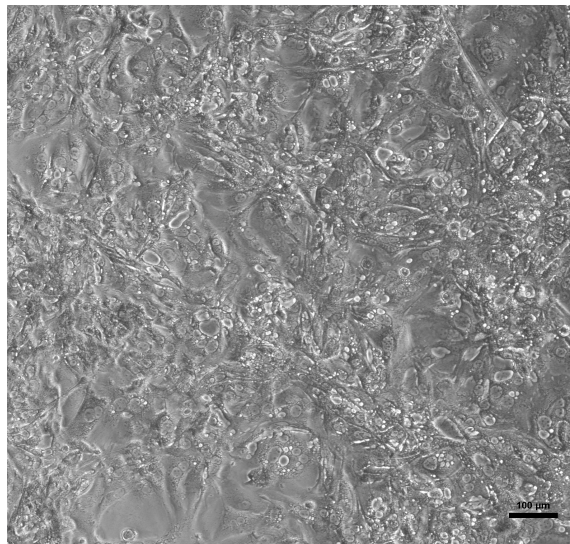
Post-thaw viability: 89.5 ± 4.9 %
Post-thaw yield per vial: $3.0 \pm 0.3 \times 10^6$ cells
Recovery: 48.8 ± 4.5 %

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

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: 7.9 ± 0.1

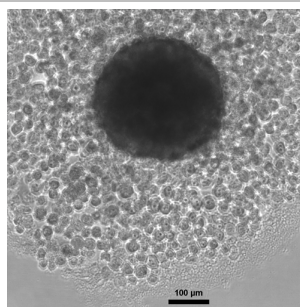
3.7

Induction with 25 µM β-Naphthoflavone

3D culture

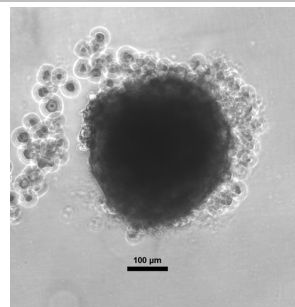
Cells seeded in 96well ULA round bottom plates (FaCellitate), 2,500 cells/well
Culture in 3D- Hepatocyte Maintenance Medium (3D-HMM)

day 7



scale bar 100 µm

day 9



scale bar 100 µm

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 HPM-Cryo):

| Time (h) | 0 | 1 | 2 | 3 | 4 | 5 |
|---------------|------------|-------------|------|------|------|------|
| Viability (%) | 89.5 ± 4.9 | 72.2 ± 48.4 | 82.7 | 73.2 | 75.0 | 76.2 |

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 | Mouse (<i>Mus musculus</i>) |
| Strain | CD-1, 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 |
| <p>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 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> | |

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| Issued by: A. Alkufairi, J. Schuldt | Verified by: K. Damrau |
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