

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

Cell Specification – Certificate of Analysis (CoA)

Lot MH231113 Batch Release: May 08, 2024

Donor data

Species: Mouse (Mus musculus) Gender: male

Strain: CD-1 Age: approx. 9 months

Cryopreservation and Thawing

Cryopreservation:

Date: Nov. 13, 2023

Amount per vial: 6.4×10^6 cells

Thawing: n=1

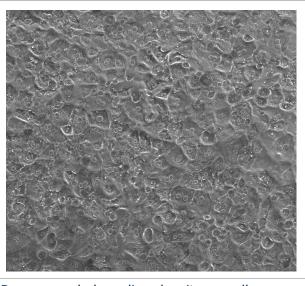
Post-thaw viability: 83.5 %

Post-thaw yield per vial: 2.0 x 10⁶ cells

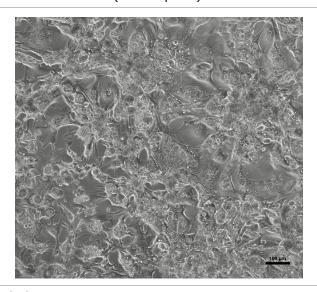
Recovery: 31.6 %

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:

Ethoxyresorufin-O-deethylation:

Induction with 25 μM β-Naphthoflavone

 $pmol/(mg \times min)$

24well: 8.5 ± 0.27

X-fold induction

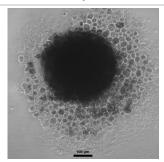
4.7



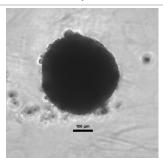
3D culture

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

day 7 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
Viability (%)	83.5	70.2	65.9	65.1	54.7

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 (Mus musculus)	
Strain	CD-1, 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 ≥ 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.

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