

BL6-MHCP-I-3D Mouse Hepatocytes Cryopreserved Plateable for Induction Assays and for 3D culture

Cell Specification – Certificate of Analysis (CoA)

Lot MH240111-1 (Pool of 4) Batch Release: February 05, 2024

Donor data

Species: Mouse (Mus musculus) Strain: Black 6 (C57BL6JR) Gender: 4 males
Age: approx. 6 months

Cryopreservation and Thawing

Cryopreservation:

Date: Jan 11, 2024 Amount per vial: 6.59 x 10⁶ cells **Thawing:** n=1

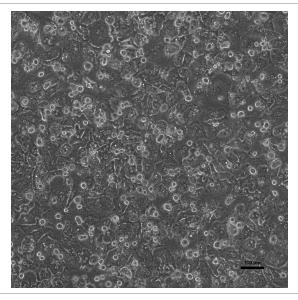
Post-thaw viability: 86.3 %

Post-thaw yield per vial: 3.46 x 10⁶ cells

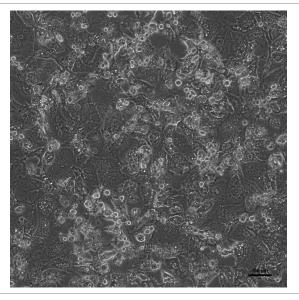
Recovery: 52.5 %

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:

Ethoxyresorufin-O-deethylation:

Induction with 25 μM β -Naphthoflavone

pmol/(mg × min)

24well: 9.3 ± 0.2

X-fold induction

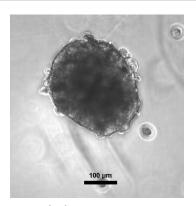
6.2



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

Suspension culture

Viability test on orbital shaker (Eppendorf Thermomixer C, 1000 rpm at 37 $^{\circ}$ C with 0.5 x 10^{6} cells in 0.5 ml HPM-Cryo):

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	86.3	75.8	81.3	78.7	77.7	71.7	66.1	65.6

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	Black 6 (C57BL6JR)			
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.