

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

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

Lot MH240110 (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:**

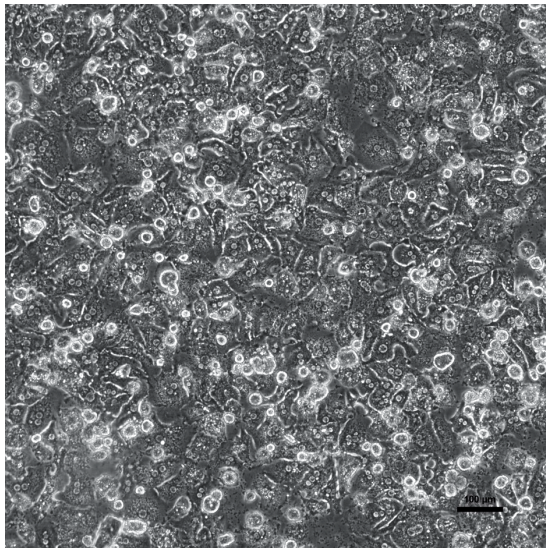
Date: Jan 10, 2024  
Amount per vial:  $5.1 \times 10^6$  cells

**Thawing:** n=1

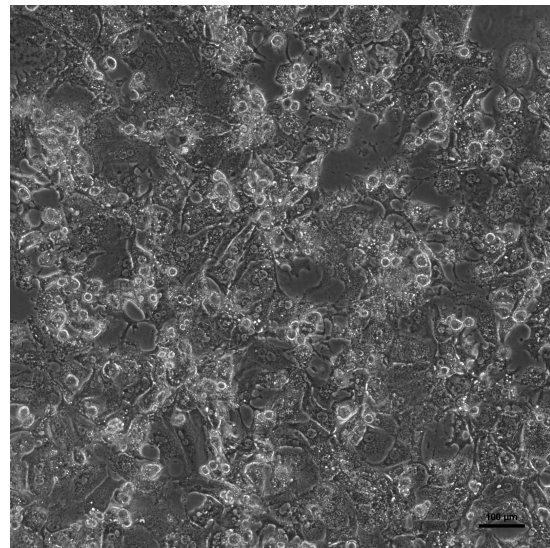
Post-thaw viability: 85.8 %  
Post-thaw yield per vial:  $1.8 \times 10^6$  cells  
Recovery: 35.3 %

**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 – 160,000 cells/well

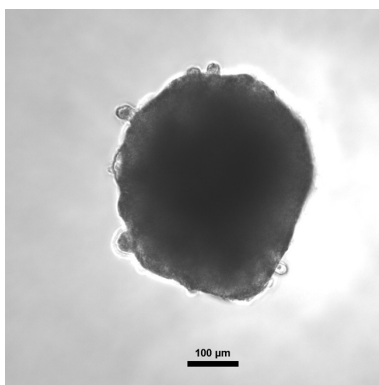
Culture in Human Hepatocyte Maintenance Medium (HHMM).

CYP P450 activity in 2D culture after thawing:	pmol/(mg × min)	X-fold induction
Ethoxyresorufin-O-deethylation:	24well: $14.3 \pm 0.2$	9.2
Induction with 25 µM β-Naphthoflavone	96well: $20.6 \pm 4.6$	3.7

### 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



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	4	5
Viability (%)	86.1	80.2	77.1	82.5	80.7	77.0	67.8	66.7

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 LN<sub>2</sub>**

This product should be considered as potential biohazard. Only intended for *in vitro* use.

<b>Detailed animal information and husbandry conditions</b>	
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
<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 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: J. Schuldt	Verified by: K. Damrau
-----------------------	------------------------