

CERTIFICATE OF ANALYSIS

Lot#: CHF2414-L-HE-Z

PRODUCT DESCRIPTION

Reference: HuHECPMI/4-
Product: Cryopreserved Human Hepatocytes
Category: Plateable, Cytochrome P450 inducible
Spheroid qualified: Yes
Organoid qualified: Yes
Specific culture requirements: No

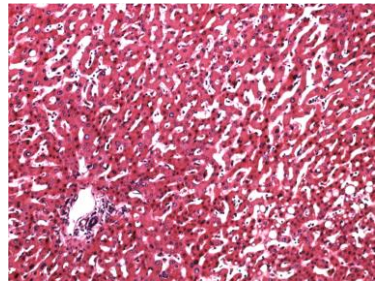
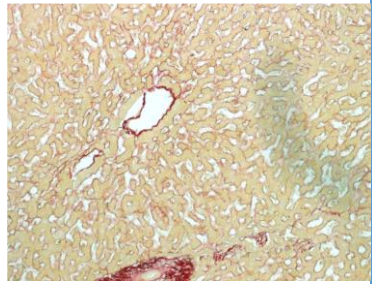
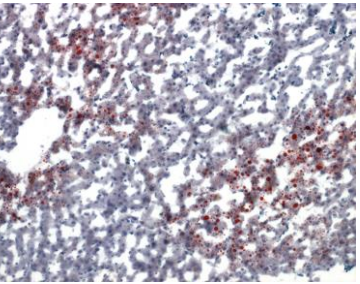
Isolation date: 30th May 2024
Initial Isolation Viability: 91.25%
Storage conditions: -196°C using LN₂
Sterility test: negative for mycoplasma, bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Female	Caucasian	65	35.81	No	No	No
Pathology			Serological Data ¹				
Metastatic tumor			Tested negative less than 3 months before surgery				

Patient informed consent was obtained. ¹The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C, and SARS-CoV-2. Donor medical history was also examined prior to accepting this donor. *For donor's medication information, please contact us.*

DONOR HISTOLOGY

Hematoxylin & Eosin staining	Sirius Red staining	Oil red staining
		

- Hematoxylin & Eosin: Liver with marked vascular congestion with enlarged sinusoidal lumens, but with no signs of hepatocyte necrosis/damage. There is observable hepatic steatosis in pericentral hepatocytes in certain areas.

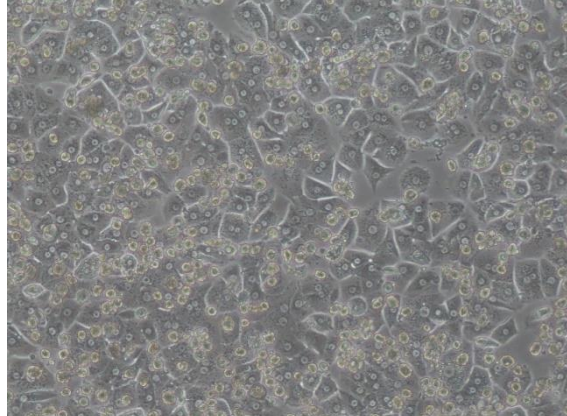

- Sirius red: Normal liver with no fibrosis detectable.

- Oil red: Liver with patchy areas of observable steatosis, generally with pericentral hepatocytes turning out positive for oil red staining.

Conclusions: The liver showed evident vascular congestion at the sinusoidal level, without any detectable fibrosis or necrosis. Disseminated patchy areas of steatotic hepatocytes.

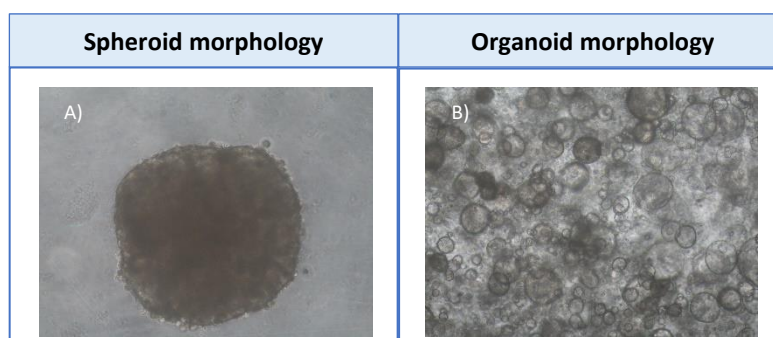
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CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells (cells/vial):	4.12x10 ⁶	± 0.91x10 ⁶	5
Post-thaw viability (%):	83.51	3.03	5
Days in culture after thaw (24w):	20	± 0.00	1
Days in culture after thaw (96w):	10	± 0.00	1
MONOLAYER ASSESSMENT² Plateable: YES			
		Confluence 24h: 85%	
Seeding density in 24 well recommended:		2.36x10 ⁵ cells/cm ²	
Seeding density in 96 well recommended:		2.50x10 ⁵ cells/cm ²	
Cell morphology 24h		Cell morphology 96h	
			

Human hepatocytes were thawed and seeded according to BeCytes Biotechnologies culture protocol. The yield and viability were determined by a trypan blue exclusion assay after the thawing process. ²Resuspended human hepatocytes from post-thaw assessment were plated in collagen-coated 24-well plates in hepatocyte plating medium. Cells were refreshed with hepatocytes maintenance medium during the first change of medium on the day of thawing. Maintenance medium was replaced in the culture every day. If images from the 96-well plates are needed, please contact us.

3D HEPATIC SPHEROID AND ORGANOID FORMATION



A) Primary human hepatocytes self-assembled into a spheroid containing 5000 cells after 5 days in culture. These hepatic spheroids were cultured for 7-15 days in ultra-low attachment (ULA) plates with our 3D Culture Maintenance Media for hepatocytes (MHM3D). B) Primary hepatocytes were validated for their capacity to generate liver organoids. After thawing the cells using BeCytes Technologies' thawing protocols and media, 150.000 hepatocytes were mixed with 50 µl of Matrigel® and cultured using the procedure described by Huch *et al.* (2014). For more information/protocols about 3D hepatic spheroids and organoids, contact us.

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INDUCTION FOR PLATEABLE CELLS

PHASE I: CYP ACTIVITIES EXPRESSED IN pmol/min/mg protein (mean ± SD)

Induction (Specific Activity)				
Enzyme	Basal Activity on day 1	Basal Activity on day 4	Induced Activity on day 4	n-Fold induction
CYP1A2	13.32 ± 0.62	4.20 ± 0.15	33.86 ± 2.10	8.07
CYP2B6	3.50 ± 0.27	2.06 ± 0.18	10.69 ± 1.91	5.19
CYP3A4	29.86 ± 0.22	43.71 ± 0.35	52.53 ± 2.10	1.20

Cryopreserved human hepatocytes were thawed and plated in 24well collagen I coated plates. Cells were overlaid with Matrigel® (Corning) in Human Hepatocyte Maintenance Medium at first medium change at day of thawing. Treatment (n=2 per compound) with vehicle control [0.15% (v/v) DMSO] or inducers (Rifampicin, β-Naphthoflavone and Phenobarbital) began 1-day post-plating and continued for 72 hours. At the end of induction, monolayers were rinsed with PBS and incubated with probe substrate solutions in culture media. See Table 1 for information on each probe substrate. Metabolites were quantified by LC-MS and normalized to protein content. The fold induction was calculated by dividing the induced activity by the vehicle basal activity on the same day in culture.

PHASE I: CYP450 mRNA induction

CYP (mRNA)	n-Fold Induction
CYP1A2	100 ± 19
CYP2B6	30 ± 5
CYP3A4	92 ± 7

Cryopreserved human hepatocytes were thawed, plated in 24well collagen I coated plates in Hepatocyte Plating Medium. Cells were overlaid with Matrigel® (Corning) in Human Hepatocyte Maintenance Medium at first medium change at day of thawing. Maintenance medium was replaced in the cultures daily. Treatment (n=2 per compound) with vehicle control [0.15% (v/v) DMSO] or inducers (Rifampicin, β-Naphthoflavone and Phenobarbital) began 1-day post-plating and continued for 72 hours. At the end of the treatment period, RNA was isolated for mRNA analysis.

Table 1. Substrates Phase I

Enzyme	Probe Substrate	Concentration (µM)	Incubation Time (min)	Metabolite
CYP1A2	Phenacetin	100	30	Acetaminophen
CYP2B6	Bupropion	500	30	Hydroxybupropion
CYP3A4	Midazolam	30	30	1-Hydroxymidazolam

PHASE II: UGTs & SULT ACTIVITIES 24h AFTER SEEDING EXPRESSED IN pmol/min/mg PROTEIN (mean ± SD)



Enzyme	Conjugate	pmol/min/mg
UGT	7-OH coumarin glucuronide	121.08 ± 2.47
SULT	7-OH coumarin sulfate	9.61 ± 0.80

Cryopreserved human hepatocytes were thawed, plated in 24well collagen I coated plates in Hepatocyte Plating Medium. Cells were overlaid with Matrigel® (Corning) in Human Hepatocyte Maintenance Medium at first medium change at day of thawing. On day 1, hepatocytes were incubated with 7-Hydroxycoumarin to assay for UDP-Glucuronosyltransferase (UGT) and Sulfotransferase (SULT) activities. See Table 2 for information on each probe substrate. Metabolites were quantified by LC-MS and normalized to protein content.

If you need help for an experiment, just contact us, our experts will be pleased to assist you

CERTIFICATION:

The viability and performance of the primary human hepatocytes provided depend primarily on the use of appropriate media and reagents, as well as the use of sterile plastics. Likewise, proper handling protocols must be followed. Please note that if these parameters are not carefully considered, the cellular response obtained in the assays may be lower than expected.

Name	Title	Signature	Cytes Biotechnologies, S.L.	Date
Pilar Sainz de la Maza	Quality Manager			24/07/24

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CELL COUNTING

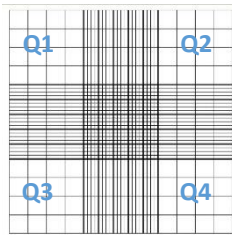
Lot #: _____

Date: ____/____/____

MORPHOLOGY

- Clear cytoplasm
- Rounded shape
- Cell swelling
- Hardly any debris
- Clear membranes
- Membrane blebbing
- Lipid droplets
- Prevalent debris

TRYPAN BLUE COUNTING RESULTS



NEUBAUER CHAMBER COUNTING					
Quadrant	Live cells	+	Dead cells	=	Total cells
Quadrant 1		+		=	
Quadrant 2		+		=	
Quadrant 3		+		=	
Quadrant 4		+		=	
Total		+		=	

VIABILITY

$$\frac{(\text{Live cells})}{(\text{Total cells})} \times 100 = \text{Viability (\%)}$$

YIELD

$$\frac{(\text{Total cells}) \times (\text{Dilution factor}) \times 10^4 \times (\text{Current volume}) \text{ ml}}{(\text{Counted quadrants})} = \text{cells (Total number of cells)}$$

**This factor (10⁴) is applicable when it is used a Hemocytometer*

SEEDING DENSITY

$$\frac{(\text{Desired number of cells})}{(\text{Total number of cells})} \times \frac{\text{cells} \times (\text{Current volume}) \text{ ml}}{\text{cells}} = \text{ml (Volume needed for your cells)}$$

Keep in mind the final volume per dish or plate to use (Volume needed) and then calculate the needed volume to add: $(\text{Total volume well}) \text{ ml} - (\text{Cells total volume}) \text{ ml} = \text{ml (Volume to add)}$

Surface of the most common plates for culture:

Brand	24-well plate	96-well plate
ThermoFisher	1.90 cm ² /well	0.32 cm ² /well
Corning®	2.00 cm ² /well	0.36 cm ² /well
Falcon®	1.90 cm ² /well	0.32 cm ² /well
Eppendorf	2.08 cm ² /well	0.37 cm ² /well

COMMENTS

COUNTED BY:

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