

## CERTIFICATE OF ANALYSIS

Lot#: CHM2225-HE-Z

### PRODUCT DESCRIPTION

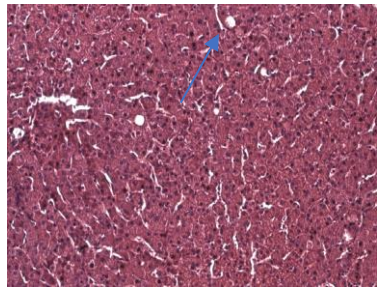
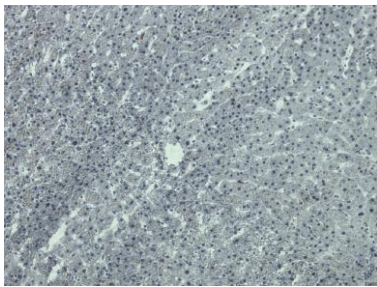
**Reference:** HuHECPMI/6+**Product:** Cryopreserved Human Hepatocytes**Category:** Plateable, Cytochrome P450 inducible**Spheroid qualified:** No**Organoid qualified:** Yes**Isolation date:** 24<sup>th</sup> October 2022**Initial Isolation Viability:** 87.24%**Storage conditions:** -196°C using LN<sub>2</sub>**Sterility test:** Negative for mycoplasma, bacteria, yeast, and fungi

### DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use	Pathology
Human	Male	Caucasian	73	28	No	No	No	Hepatocellular Carcinoma
HLA typing <sup>1</sup>				Serological Data <sup>2</sup>				
A02, A02, B51, B39, C07, C08				Tested negative less than 3 months before surgery				

Patient informed consent was obtained. <sup>1</sup>HLA typing is analyzed by HLA-HD v1.5.0 software. HLA allele dictionary is available at the IPD-IMGT/HLA database. <sup>2</sup>The 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 more information about HLA typing and donor's medication, please contact us.*

### DONOR HISTOLOGY

Hematoxylin & Eosin staining	Sirius Red staining	Oil red staining
		

- Hematoxylin & Eosin: Parenchyma with only very few isolated steatotic hepatocytes present, no detectable necrosis, and apparent healthy tissue. Also, no signs of fibrotic areas present in this liver.

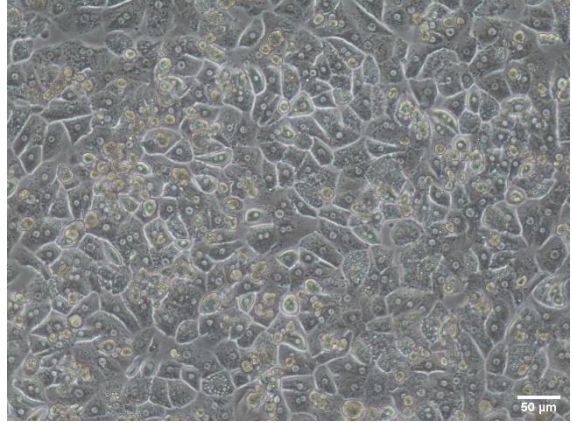
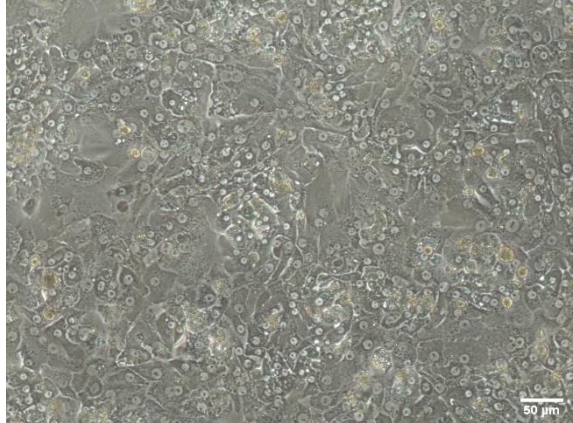
- Sirius red: Liver with no noticeable signs of fibrosis, with only very mild accumulation of sirius red staining in portal areas. Very little to none matrix deposition in the sinusoidal areas close to periportal space.

- Oil red: Very few areas with "fatty vacuolation" with oil red in hepatocytes. Light steatosis.

**Conclusions:** Liver with very little to absence of fibrotic tissue present. Most of the tissue seems normal without any fat vacuolated hepatocytes.

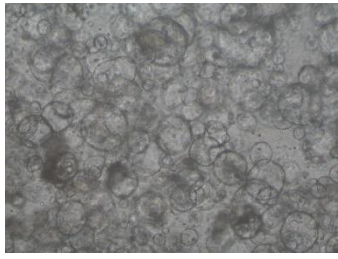
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### CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells (cells/vial):	9.36x10 <sup>6</sup>	± 1.09x10 <sup>6</sup>	8
Post-thaw viability (%):	86.29	± 2.44	8
Days in culture after thaw (24w):	12	± 0.0	1
<b>MONOLAYER ASSESSMENT<sup>3</sup> Plateable: YES</b>			
<b>Seeding density in 24 well recommended:</b>		<b>Confluence 24h: 90%</b>	
		2.10x10 <sup>5</sup> cells/cm <sup>2</sup>	
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. <sup>3</sup>Resuspended 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.

### 3D HEPATIC SPHEROID AND ORGANOID FORMATION

Spheroid morphology	Organoid morphology
This lot is not suitable for 3D spheroid culture according to BeCytes Technologies protocols	

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 organoids, contact us.

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## INDUCTION FOR PLATEABLE CELLS

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

Enzyme	Induction (Specific Activity)			n-Fold induction
	Basal Activity on day 1	Basal Activity on day 4	Induced Activity on day 4	
CYP1A2	3.11 ± 0.07	1.30 ± 0.10	15.55 ± 0.19	11.99
CYP2B6	1.34 ± 0.05	0.40 ± 0.01	2.11 ± 0.12	5.27
CYP3A4	3.12 ± 0.17	3.45 ± 0.21	15.94 ± 0.16	4.62

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	27 ± 10
CYP2B6	21 ± 9
CYP3A4	13 ± 5

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	75.30 ± 3.07
SULT	7-OH coumarin sulfate	36.54 ± 0.81

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.

Table 2. Substrates Phase II

Enzyme	Probe Substrate	Concentration (µM)	Incubation Time (min)	Metabolite
UGT	7-Hydroxycoumarin	100	30	7-Hydroxycoumarin-glucuronide
SULT	7-Hydroxycoumarin	100	30	7-Hydroxycoumarin-sulfate

## GENOTYPING DATA

CYP Enzyme Genotyping					
Gene	rs Number	Result	Allele Freq. %	Ref. Allele	cDNA Ref. seq.
CYP1A2	rs12720461	C/C	-	C	-
CYP1A2	rs2069526	T/T	-	T	-
CYP1A2	rs2470890	T/C	C=1	T	c.1548T>C
CYP1A2	rs35694136	T/T	-	T	-

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CYP1A2	rs56107638	G/G	-	G	G
CYP1A2	rs56276455	G/G	-	G	G
CYP1A2	rs72547515	G/G	-	G	G
CYP1A2	rs72547517	G/G	-	G	G
CYP1A2	rs762551	C/A	A=1	C	-
CYP1A2*11	rs72547513	C/C	-	C	C
CYP1A2*1C	rs2069514	G/G	-	G	-
CYP1A2*4	rs72547516	A/A	-	A	A
CYP1A2*5	rs55889066	G/G	-	G	G
CYP1A2*6	rs28399424	C/C	-	C	C
CYP2B6	rs2279343	A/G	G=0.377551	A	c.785A>G
CYP2B6	rs28399499	T/T	-	T	T
CYP2B6	rs3211371	C/C	-	C	C
CYP2B6	rs34097093	C/C	-	C	C
CYP2B6	rs34223104	T/T	-	T	-
CYP2B6	rs3745274	G/T	T=0.421053	G	c.516G>T
CYP2B6	rs8192709	C/C	-	C	C
CYP2B6*11	rs35303484	A/A	-	A	A
CYP2C19*10	rs6413438	C/C	-	C	C
CYP2C19*17	rs12248560	C/C	-	C	-
CYP2C19*2	rs4244285	G/G	-	G	G
CYP2C19*2B	rs17878459	G/G	-	G	G
CYP2C19*3	rs4986893	G/G	-	G	G
CYP2C19*4	rs28399504	A/A	-	A	A
CYP2C19*5	rs56337013	C/C	-	C	C
CYP2C19*6	rs72552267	G/G	-	G	G
CYP2C19*7	rs72558186	T/T	-	T	T
CYP2C19*8	rs41291556	T/T	-	T	T
CYP2C19*9	rs17884712	G/G	-	G	-
CYP2C8*2	rs11572103	T/T	-	T	T
CYP2C8*3	rs10509681	T/C	C=0.619048	T	c.890A>G
CYP2C9*10	rs9332130	A/A	-	A	A
CYP2C9*11	rs28371685	C/C	-	C	C
CYP2C9*13	rs72558187	T/T	-	T	T
CYP2C9*15	rs72558190	C/C	-	C	C
CYP2C9*2	rs1799853	C/T	T=0.453061	C	c.430C>T
CYP2C9*27	rs7900194T	G/G	-	G	G
CYP2C9*3	rs1057910	A/A	-	A	A
CYP2C9*4	rs56165452	T/T	-	T	T
CYP2C9*5	rs28371686	C/C	-	C	C
CYP2C9*52	rs72558192	A/A	-	A	A
CYP2C9*6	rs9332131	A/A	-	A	A
CYP2C9*7	rs67807361	C/C	-	C	C
CYP2C9*8_c.449G>A	rs7900194A	G/G	-	G	G
CYP2C9*9	rs2256871	A/A	-	A	A
CYP2D6	rs1058164	C/G	G=0.48125	C	c.408G>C
CYP2D6	rs1065852	G/G	-	G	G
CYP2D6	rs1135840	C/G	G=0.590244	C	c.1304G>C
CYP2D6	rs201377835	C/C	-	C	-
CYP2D6	rs28371706	G/G	-	G	G
CYP2D6	rs28371725	C/C	-	C	-
CYP2D6	rs5030655	A/A	-	A	A
CYP2D6	rs5030862	C/C	-	C	C
CYP2D6	rs5030865A	C/C	-	C	C
CYP2D6	rs5030865T	C/C	-	C	C
CYP2D6	rs59421388	C/C	-	C	C
CYP2D6	rs61736512	C/C	-	C	C

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CYP2D6	rs769258	C/T	T=0.464789	C	c.31G>A
CYP2D6*2	rs16947	G/A	A=0.565693	G	c.733C>T
CYP2D6*2A	rs1080985	G/G	-	G	-
CYP2D6*3	rs35742686	T/T	-	T	T
CYP2D6*4	rs3892097	C/C	-	C	-
CYP2D6*7	rs5030867	T/T	-	T	T
CYP2D6*9	rs5030656	T/T	-	T	T
CYP3A4	rs12721629	G/G	-	G	G
CYP3A4	rs4646438	T/T	-	T	T
CYP3A4*13	rs4986909	G/G	-	G	G
CYP3A4*15	rs4986907	C/C	-	C	C
CYP3A4*17	rs4987161	A/A	-	A	A
CYP3A4*1B	rs2740574	C/C	-	C	-
CYP3A4*2	rs55785340	A/A	-	A	A
CYP3A4*20	rs67666821	T/T	-	T	T
CYP3A4*22	rs35599367	G/G	-	G	-
CYP3A4*3	rs4986910	A/A	-	A	A
CYP3A5	rs15524	A/A	-	A	A
CYP3A5	rs200579169	C/C	-	C	C
CYP3A5	rs28383468	G/G	-	G	G
CYP3A5*10	rs41279854	A/A	-	A	A
CYP3A5*2	rs28365083	G/G	-	G	G
CYP3A5*3	rs776746	T/C	C=1	T	-
CYP3A5*6	rs10264272	C/C	-	C	C
CYP3A5*7	rs41303343	A/A	-	A	-
CYP3A5*8	rs55817950	G/G	-	G	G
CYP3A5*9	rs28383479	C/C	-	C	C

#### Phase II and Transporter Enzyme Genotyping

Gene	rs Number	Result	Allele Freq. %	Ref. Allele	cDNA Ref. seq.
ABCB1	rs1045642	A/G	G=0.99359	A	c.3435T>C
ABCG2	rs2231142	G/G	-	G	G
COMT	rs4680	G/G	-	G	G
DPYD*10	rs1801268	C/C	-	C	C
DPYD*9A	rs1801265	A/G	G=0.635294	A	c.85T>C
DPYD*9B	rs1801267	C/C	-	C	C
MTHFR_A1298C	rs1801131	T/T	-	T	T
MTHFR_C677T	rs1801133	G/A	A=0.427861	G	c.788C>T
OPRM1	rs1799971	A/A	-	A	A
SLCO1B1*1B	rs2306283	A/G	G=1	A	c.388A>G
SLCO1B1*5	rs4149056	T/T	-	T	T
TPMT*2	rs1800462	C/C	-	C	C
TPMT*3B	rs1800460	C/C	-	C	C
TPMT*3C	rs1142345	T/T	-	T	T
TPMT*4	rs1800584	C/C	-	C	-
UGT1A1*6	rs4148323	G/G	-	G	G
UGT2B15	rs1902023	A/C	C=1	A	c.253T>G
UGT2B7	rs28365063	A/A	-	A	A
VKORC1	rs2359612	A/A	-	A	-
VKORC1	rs2884737	A/A	-	A	-
VKORC1	rs7294	C/C	-	C	-
VKORC1	rs8050894	C/G	G=1	C	-
VKORC1	rs9923231	C/T	T=1	C	-
VKORC1	rs9934438	G/A	A=1	G	-
PNPLA3	rs738409	C/G	G=0.493902	C	c.444C>G
PNPLA3	rs2281135	G/G	-	G	-
HSD17B13	rs72613567	A/A	-	A	-

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MARC1_	rs2642438	A/G	G=1	A	c.493A>G
TM6SF2	rs58542926	C/T	T=0.521739	C	c.499G>A
GCKR	rs1260326	T/T	-	T	T
MBOAT7	rs641738	T/C	C=0.545455	T	c.50A>G

Genomic DNA was isolated from Primary Human Hepatocytes with an in-house extraction protocol and sequenced using Agilent v6 (SureSelectXT Reagent Kit+Agilent SureSelect Human All ExonV6 - Cat N. G9611B) and Novaseq 6000 PE150 strategy, reference genome hg38. Alignment was performed using BWA v0.7.17. The SNPs and InDels detection were carried out using GATK v4.0 and annotation was performed with ANNOVAR 2015Dec14.

**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			25/09/24

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### CELL COUNTING

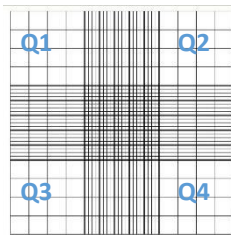
Lot #: \_\_\_\_\_

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		+		=	
<b>Total</b>		+		=	

#### 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 (10<sup>4</sup>) is applicable when it is used a Hemocytometer*

#### SEEDING DENSITY

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

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 <sup>2</sup> /well	0.32 cm <sup>2</sup> /well
Corning®	2.00 cm <sup>2</sup> /well	0.36 cm <sup>2</sup> /well
Falcon®	1.90 cm <sup>2</sup> /well	0.32 cm <sup>2</sup> /well
Eppendorf	2.08 cm <sup>2</sup> /well	0.37 cm <sup>2</sup> /well

#### COMMENTS

COUNTED BY:

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