CYTES BIOTECHNOLOGIES S.L.

Updated on: 23rd March 2023

CERTIFICATE OF ANALYSIS

Lot#: CHM2225-HE-Z

PRODUCT DESCRIPTION

Reference: HuHECPMI/6+
Product: Cryopreserved Human Hepatocytes
Category: Plateable, Cytochrome P450 inducible
Spheroid qualified: No
(see details below: 3D Spheroid formation section)

Isolation date: 24th October 2022
Initial Isolation Viability: 87.24%
Storage conditions: -196°C using LN_2
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[1]	Serological Data[2]
A02, A02, B51, B39, C07, C08	Tested negative less than 3 months before surgery

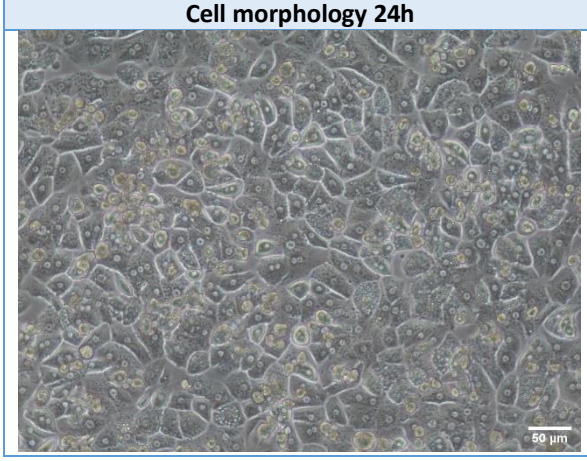
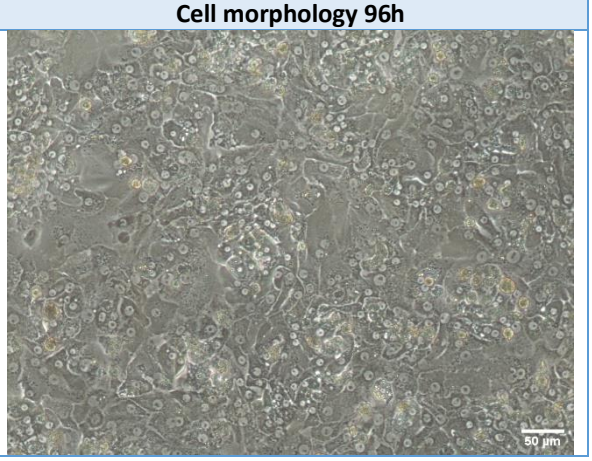
Patient informed consent was obtained. [1]HLA typing is analyzed by HLA-HD v1.5.0 software. HLA allele dictionary is available at the IPD-IMGT/HLA database. [2]The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C, and SARS-CoV-2. Donor medical history was also examined prior to accepting this donor. *For more information about HLA typing and donor's medication, please contact us.*

CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells (cells/vial):	$8.75x10^6$	± $1.52x10^6$	3
Post-thaw viability (%):	87.54	± 3.93	3
Days in culture after thaw (24w):	12	± 0.0	1
Days in culture after thaw (96w):	10	± 0.00	1

MONOLAYER ASSESSMENT[3] **Plateable: YES** **Confluence 24h:** 90%

Seeding density in 24 well recommended: $2.10x10^5$ cells/cm^2
Seeding density in 96 well recommended: $1.87x10^5$ cells/cm^2

Cell morphology 24h	Cell morphology 96h
	

Human hepatocytes were thawed and seeded according to Cytes Biotechnologies culture protocol. The yield and viability were determined by a trypan blue exclusion assay after the thawing process. [3]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

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medium at first 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.

TO OBTAIN THE RESULTS DESCRIBED ABOVE, THE CELLS OF THIS LOT MUST BE CENTRIGUFED AT 100G FOR 10 MIN AT RT

3D SPHEROID FORMATION

Spheroid morphology
Cytes **does not guarantee** that these primary hepatocytes will be suitable for 3D culture and creation of spheroid structures while using Cytes protocols.

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	5.19 ± 0.11	2.14 ± 0.17	25.73 ± 0.40	12.05
CYP2B6	2.24 ± 0.08	0.66 ± 0.01	3.48 ± 0.19	5.30
CYP3A4	5.21 ± 0.29	5.68 ± 0.34	26.37 ± 0.18	4.64

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	125.49 ± 4.92
SULT	7-OH coumarin sulfate	60.90 ± 1.44

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

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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	-
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

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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
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

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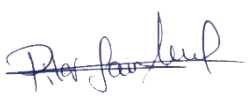
VKORC1	rs2359612	A/A	-	A	-
VKORC1	rs2884737	A/A	-	A	-
VKORC1	rs7294	C/C	-	C	-
VKORC1	rs8050894	C/G	G=1	C	-
VKORC1	rs9923231	C/T	T=1	C	-
VKORC1	rs9934438	G/A	A=1	G	-
PNPLA3	rs738409	C/G	G=0.493902	C	c.444C>G
PNPLA3	rs2281135	G/G	-	G	-
HSD17B13	rs72613567	A/A	-	A	-
MARC1_	rs2642438	A/G	G=1	A	c.493A>G
TM6SF2	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

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	Tittle	Signature	Cytes Biotechnologies, S.L.	Date
Pilar Sainz de la Maza	Quality Manager			23/03/23

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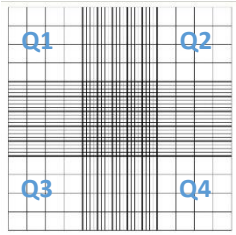
CELL COUNTING

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

$$\frac{(Live\ cells)}{(Total\ cells)} x100 = \quad Viability\ (\%)$$

YIELD

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

**This factor (10^4) is applicable when it is used a Hemocytometer*

SEEDING DENSITY

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

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

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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