Updated on: 24th March 2023

CERTIFICATE OF ANALYSIS

Lot#: CyHum(f)19009-HE-C

PRODUCT DESCRIPTION

Reference: HuHECPMI/6+
Product: Cryopreserved Human Hepatocytes
Category: Plateable, Cytochrome P450 inducible
Spheroid qualified: Yes
(see details below: 3D Spheroid formation section)

Isolation date: 21st November 2019
Initial Isolation Viability: 88.00%
Storage conditions: -196°C using LN_2
Sterility test: negative for bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Female	Caucasian	70	18	No	No	No

Pathology	Serological Data[1]
M1 Hepatic	Tested negative less than 3 months before surgery Epstein Bar positive

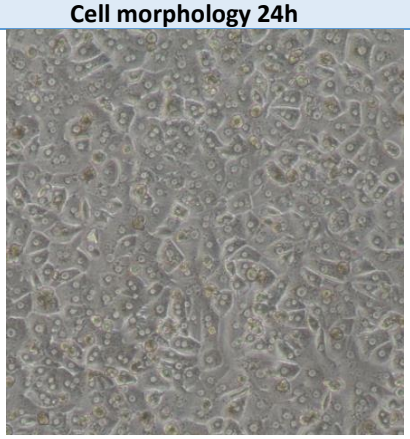
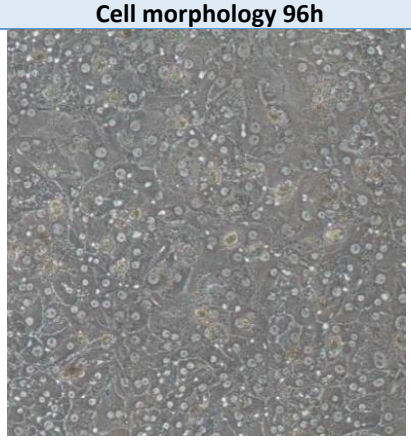
Patient informed consent was obtained. [1]The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C. Donor medical history was also examined prior to accepting this donor. *For donor's medication information, please contact us.*

CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells (cells/vial):	$7.02x10^6$	± $2.74x10^6$	6
Post-thaw viability (%):	91.55	± 2.75	6
Days in culture after thaw (24w):	24	± 0.00	2
Days in culture after thaw (96w):	5	± 0.00	2

MONOLAYER ASSESSMENT[2] **Plateable: YES** **Confluence:** 95%

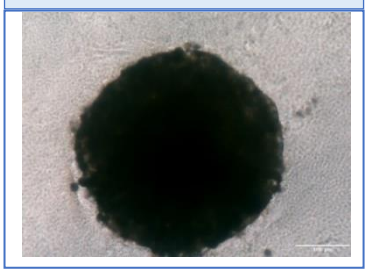
Seeding density in 24 well recommended: $2.12x10^5$ cells/cm^2
Seeding density in 96 well recommended: $2.20x10^5$ cells/cm^2

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. [2]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. If images from the 96-well plates are needed, please contact us.

3D SPHEROID FORMATION

Spheroid morphology



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). *For more information/protocols about 3D hepatocyte spheroids, contact us.*

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	19.65 ± 1.79	15.89 ± 0.69	207.41 ± 15.22	13.06
CYP2B6	20.36 ± 0.62	22.42 ± 6.04	64.17± 10.65	2.86
CYP3A4	31.50 ± 1.51	50.02 ± 3.07	157.21 ± 12.21	3.14

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	15
CYP2B6	12
CYP3A4	8

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	244.91 ± 21.79
SULT	7-OH coumarin sulfate	43.96 ± 1.05

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

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			24/03/23

BeCYTES BIOTECHNOLOGIES

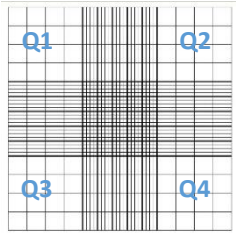
CELL COUNTING

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

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

YIELD

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

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

SEEDING DENSITY

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

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

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: