Updated on:29th November 2023

CERTIFICATE OF ANALYSIS

Lot#: CHF2304-HE-Z

PRODUCT DESCRIPTION

Reference: HuHECSM/4-
Product: Cryopreserved Human Hepatocytes
Category: Suspension, Metabolism certified
Spheroid qualified: No
(see details below: 3D Spheroid formation section)

Isolation date: 17th April 2023
Initial Isolation Viability: 96.83%
Storage conditions: -196°C using LN_2
Sterility test: negative for bacteria, yeast, and fungi

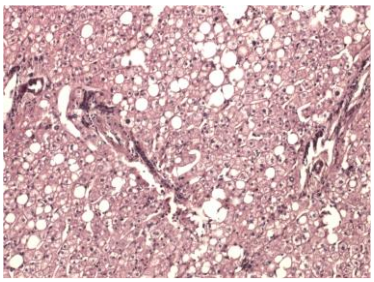
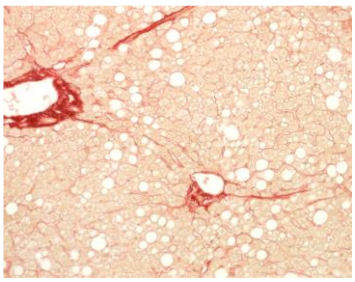
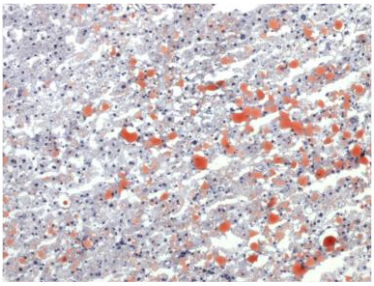
DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Female	Caucasian	55	25.39	No	No	No

Pathology	Serological Data[1]
Metastatic Tumor	Tested negative less than 3 months before surgery

Patient informed consent was obtained. [1]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 donor's medication information, please contact us.*

DONOR HISTOLOGY

Hematoxylin & Eosin staining	Sirius Red staining	Oil red staining
		

- Hematoxylin & Eosin: Liver with some debris/cells are observable inside some large vessels (orange arrow). Parenchyma with macrovesicular steatosis present (yellow arrows) and detectable necrosis (anuclear hepatocytes) throughout the parenchyma.

- Sirius red: Liver with very light signs of fibrosis, with only very discrete accumulation of sirius red staining in portal areas and discrete bridging fibrosis. Very little matrix deposition in the sinusoids close to periportal areas.

- Oil red: Extensive "fatty vacuolation" with oil red in hepatocytes showing macrovesicular steatosis and ballooning degeneration of hepatocytes throughout the whole liver.

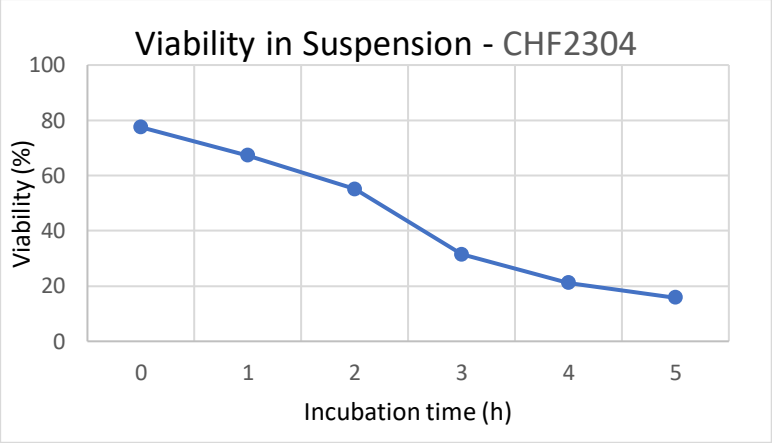
Conclusions: Liver with complete washing at the sinusoidal level, with very light areas with matrix deposition present. Most of the tissue with macrovesicular steatosis and ballooning degeneration.

CHARACTERIZATION FOR SUSPENSION CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells/vial:	$4.52x10^6$	± $0.61x10^6$	5
Viability (%):	77.85	± 7.02	5

Human hepatocytes were thawed according to BeCytes Biotechnologies protocol. The post-thawing yield and viability (trypan blue exclusion assay) of hepatocytes were assessed after a purification process.

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	77.49	66.20	67.30	46.14	55.07	31.40	21.18	15.75
SD	± 0.00	± 3.80	± 2.44	± 1.32	± 1.91	± 3.74	± 6.09	± 1.95



Resuspended human hepatocytes suspension (0,5 * 10^6 cells in 0.5 ml medium) from the post-thaw assessment were incubated up to 5 h at 37°C. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1000 rpm) using Eppendorf Thermomixer C. In the first two hours, samples were taken at every 30 min, after 2 h samples were taken at every 60 min. At each time point the viability of cells was calculated.

3D SPHEROID FORMATION

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

ENZYME ACTIVITY IN SUSPENSION CELLS

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

	Enzyme Activity
Enzyme	Activity (pmol/min/mg protein)
CYP1A2	13.21 ± 0.05
CYP2B6	12.00 ± 1.62
CYP3A4/5	5.79 ± 0.39

Cryopreserved human hepatocytes in suspension culture (0.5 *10^6 cells in 0.5 ml medium) were incubated with specific substrates (see table below) for 30 min at 37 °C for determination of CYP activities. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1.000 rpm) in Eppendorf Thermomixer C. Metabolites were quantified by LC-MS and normalized to protein content. The substrates were applied as cocktail for simultaneous assessment of CYP 450 activity. Results are expressed in pmol/mg*min.

Substrates Phase I

Enzyme	Substrate	Concentration (µM)	Incubation Time (min)	Metabolite
CYP1A2	Phenacetin	100	30	Acetaminophen
CYP2B6	Bupropion	500	30	Hydroxybupropion
CYP3A4/5	Midazolam	3	30	1-Hydroxymidazolam

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			29/11/23