

CERTIFICATE OF ANALYSIS

Lot#: CHF2224-HE-Z

PRODUCT DESCRIPTION

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

Isolation date: 17th October 2022
Initial Isolation Viability: 88.5%
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	59	29.72	No	No	No
Pathology		Serological Data ¹					
Adenocarcinoma		Tested negative less than 3 months before surgery					

Patient informed consent was obtained. ¹The donor was serologically tested negative for the 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: Parenchyma with extensive steatosis present (yellow arrows), detectable necrosis (anuclear hepatocytes), and ductular reaction/small hepatocytes present (green arrow) 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 bridging fibrosis. Very little to none matrix deposition in the sinusoidal areas close to periportal space.

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

Conclusions: Liver with very little to absence of fibrotic tissue present. Most of the tissue with macrovesicular steatosis

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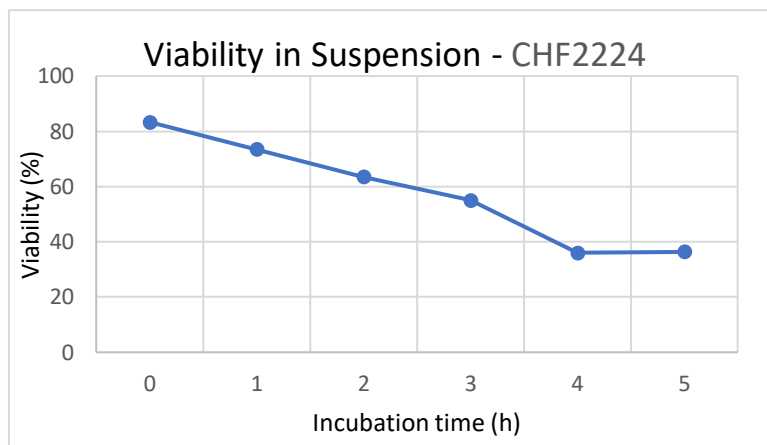


CHARACTERIZATION FOR SUSPENSION CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells/vial:	2.30x10 ⁶	± 0.85x10 ⁶	6
Viability (%):	83.16	± 7.41	6

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 (%)	83.33	75.96	73.42	72.85	63.46	55.08	36.05	36.43
SD	± 8.10	± 0.96	± 1.24	± 0.08	± 0.18	± 2.05	± 5.44	± 1.81



Resuspended human hepatocytes suspension (0,5 * 10⁶ 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.

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			14/10/24

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