

CERTIFICATE OF ANALYSIS

Lot#: CHM2215-HE-C

PRODUCT DESCRIPTION

Reference: HuHECS/4+
Product: Cryopreserved Human Hepatocytes
Category: Suspension
Spheroid qualified: NO
(see details below: 3D Spheroid formation section)

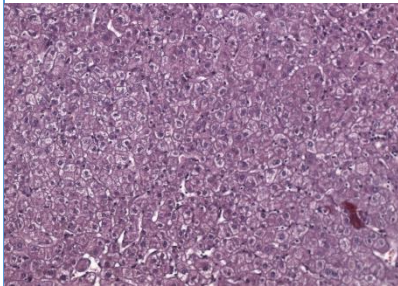
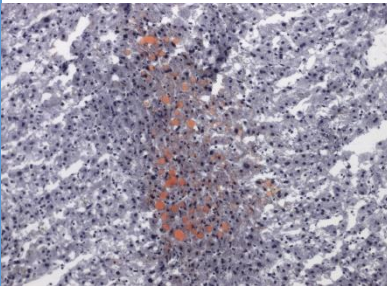
Isolation date: 13th June 2022
Initial Isolation Viability: 74.25%
Storage conditions: -196°C using LN₂
Sterility test: negative for bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	69	27.18	No	No	No
Pathology			Serological Data ¹				
Metastatic tumor			Tested negative less than 3 months before surgery				

Patient informed consent was obtained. ¹The 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: Very few areas of the parenchyma with large vacuolated hepatocytes and significant hepatocellular ballooning (estimated hepatic steatosis much less than 30%) and no detectable necrosis. 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 matrix deposition in the sinusoidal areas and increased deposition in periportal areas.

- Oil red: Very few areas with "fatty" hepatocytes, but with hepatocyte ballooning present, showing very discrete areas of necrosis.

Conclusions: Liver with limited areas with "fatty and ballooned" hepatocytes, and very little fibrotic tissue present. Most of the tissue seems normal.

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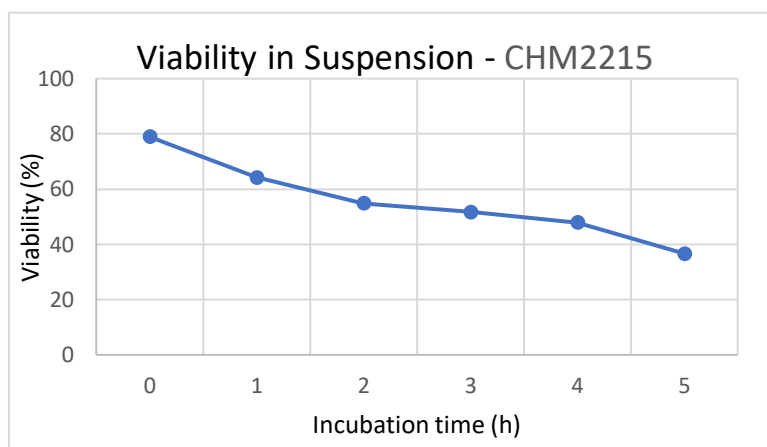


CHARACTERIZATION FOR SUSPENSION CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells/vial:	5.73×10^6	$\pm 0.77 \times 10^6$	4
Viability (%):	81.10	± 2.43	4

Human hepatocytes were thawed according to Cytes 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 (%)	78.91	65.89	64.26	64.96	54.84	51.70	47.95	36.69
SD	± 0.00	± 0.34	± 1.31	± 0.30	± 0.49	± 1.12	± 4.54	± 4.09



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

Cytes **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			31/03/23

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