

CERTIFICATE OF ANALYSIS

Lot#: CHF2217-HE-C

PRODUCT DESCRIPTION

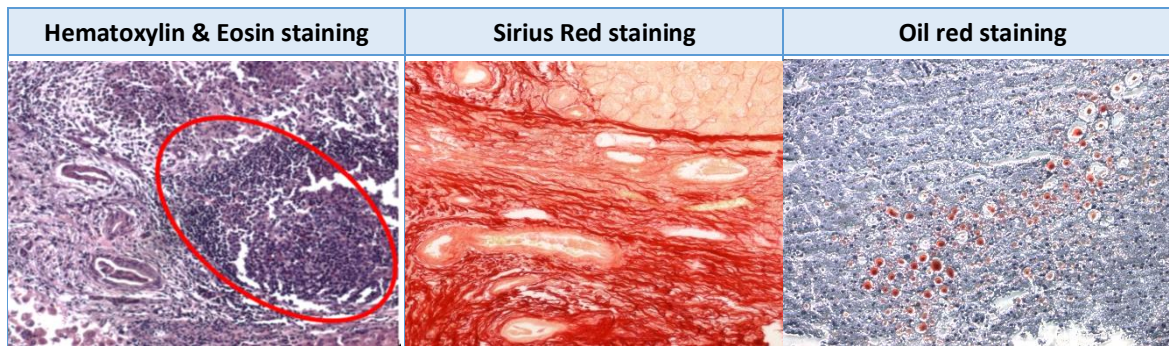
Reference: HuHECSM/4+**Product:** Cryopreserved Human Hepatocytes**Category:** Suspension, Metabolism certified**Spheroid qualified:** No*(see details below: 3D Spheroid formation section)***Isolation date:** 4th July 2022**Initial Isolation Viability:** 65.61%**Storage conditions:** -196°C using LN₂**Sterility test:** negative for bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Female	Caucasian	76	32.81	No	No	No
Pathology			Serological Data ¹				
Hepatocellular Carcinoma, NASH diagnosis			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: Small and discrete areas of the parenchyma with large vacuolated hepatocytes and significant hepatocellular ballooning (estimated hepatic steatosis much less than 30%) and detectable necrosis (green arrows). Evidence of hepatic proliferation in periportal areas (eosinophilic small hepatocytes) probably due to increased hepatocyte turnover. Granuloma tissue (red ellipse) present in periportal areas and other areas of the parenchyma showing hepatic inflammation.

- Sirius red: Liver with noticeable fibrosis with fibrotic bridges between periportal areas, showing septal formation between acinar units. Little matrix deposition in the sinusoidal areas and increased deposition in pericentral areas.

- Oil red: Small areas with "fatty" hepatocytes, but with hepatocyte ballooning present, showing areas of necrosis and absence of parenchymal cells.

Conclusions: Liver with incomplete washing, with limited areas with "fatty and ballooned" hepatocytes present, necrosis and significant septal bridges of fibrotic tissue between acinar units. By the size of the fibrotic tissue, this seems to be an F2-F3 liver.

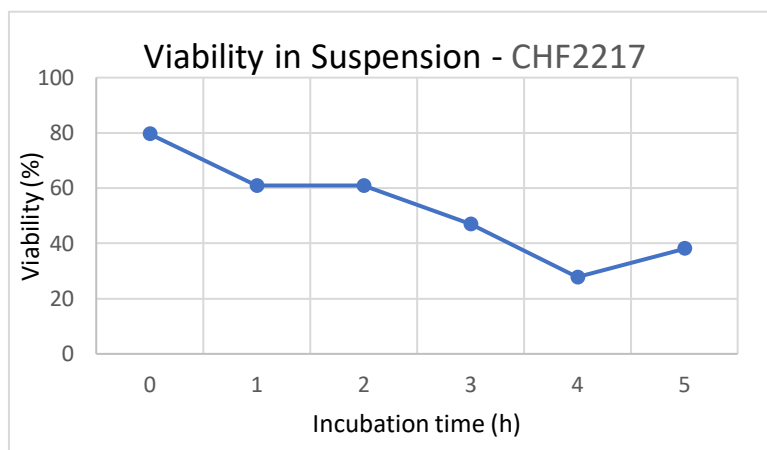
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CHARACTERIZATION FOR SUSPENSION CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells/vial:	5.74x10 ⁶	± 0.70x10 ⁶	3
Viability (%):	79.58	± 2.92	3

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 (%)	79.58	63.54	60.91	64.18	60.94	46.96	27.80	38,09
SD	± 4.12	± 9.06	± 2.00	± 6.21	± 2.21	± 8.01	± 0.29	± 0.00



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.

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	34.93 ± 2.17
CYP2B6	23.94 ± 1.89
CYP3A4/5	8.53 ± 0.70

Cryopreserved human hepatocytes in suspension culture (0.5 * 10⁶ 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

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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			27/02/24

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