

CERTIFICATE OF ANALYSIS

Lot#: CHF2204-HE-Z

PRODUCT DESCRIPTION

Reference: HuHECSM/4-
Product: Cryopreserved Human Hepatocytes
Category: Suspension, Metabolism certified
Spheroid qualified: No
Organoid qualified: Yes

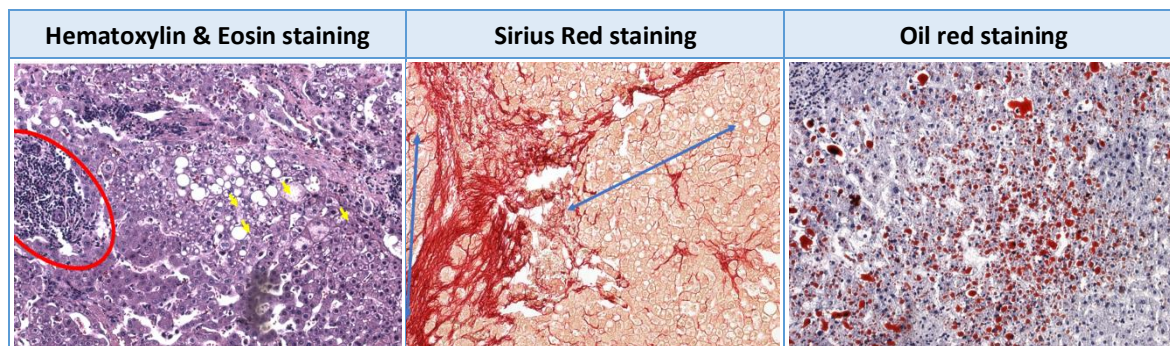
Isolation date: 11th February 2022
Initial Isolation Viability: 85%
Storage conditions: -196°C using LN₂
Sterility test: negative for mycoplasma, bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Female	Caucasian	68	33.12	No	No	No
Pathology			Serological Data ¹				
Cholangiocarcinoma, 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: Large areas of the parenchyma with vacuolated hepatocytes and significant hepatocellular ballooning (estimated hepatic steatosis >30%) and manifest centrilobular necrosis (yellow 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, compatible with NASH.

- Sirius red: Large fibrotic septa in periportal areas bridging portal triads with extensive matrix deposition in sinusoidal structures. By the bridging fibrosis in this tissue, it is scored as a F3 in the NASH CRN score.

- Oil red: Extensive and widespread fat filled hepatocytes with clear hepatocellular ballooning (>30% of steatosis). Granuloma tissue is also evident in these histological sections (red ellipse).

Conclusions: Evident and extensive bridging fibrosis between portal triads. Widespread lipid accumulation in most of the hepatocytes with visible hepatic necrosis. Zones of granuloma tissue compatible with liver inflammation (NASH).

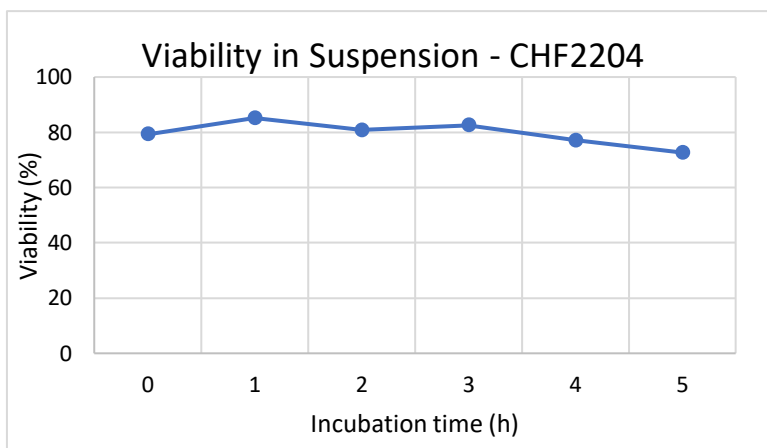
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CHARACTERIZATION FOR SUSPENSION CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells/vial:	3.68x10 ⁶	± x10 ⁶	8
Viability (%):	74.90	± 8.84	8

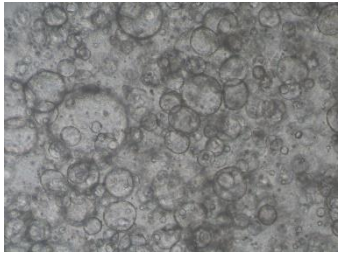
Human hepatocytes were thawed according to BeCytes Biotechnologies protocol. The post-thawing yield and viability (trypan blue exclusion assay) of hepatocytes were assessed after a purification process.

Time (h)	0	0.5	1	1.5	2	3	4	5
Viability (%)	79.32	87.6	85.17	89.72	80.85	82.52	77.14	72.66
SD	± 2.09	± 0.14	± 0.47	± 2.24	± 5.56	± 2.96	± 4.04	± 0.35



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 HEPATIC SPHEROID AND ORGANOID FORMATION

Spheroid morphology	Organoid morphology
This lot is not suitable for 3D spheroid culture according to BeCytes Technologies protocols	

Primary hepatocytes were validated for their capacity to generate liver organoids. After thawing the cells using BeCytes Technologies' thawing protocols and media, 150.000 hepatocytes were mixed with 50 µl of Matrigel® and cultured using the procedure described by Huch *et al.* (2014). For more information/protocols about 3D hepatic organoids, contact us.

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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	21.61 ± 1.04
CYP2B6	2.97 ± 0.12
CYP3A4/5	6.08 ± 0.19

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	Title	Signature	Cytes Biotechnologies, S.L.	Date
Pilar Sainz de la Maza	Quality Manager			25/09/24

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