

# CERTIFICATE OF ANALYSIS

Lot#: CHM2215-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:** 13<sup>th</sup> June 2022  
**Initial Isolation Viability:** 74.25%  
**Storage conditions:** -196°C using LN<sub>2</sub>  
**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 <sup>1</sup>				
Metastatic tumor			Tested negative less than 3 months before surgery				

Patient informed consent was obtained. <sup>1</sup>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: 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.**

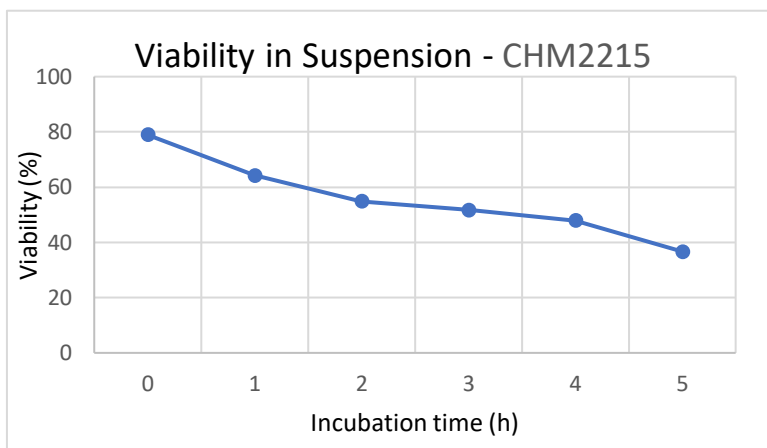
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## CHARACTERIZATION FOR SUSPENSION CELLS

Post Thaw Lot information	Result	SD	n
<b>Number of viable cells/vial:</b>	5.60x10 <sup>6</sup>	± 0.65x10 <sup>6</sup>	5
<b>Viability (%):</b>	80.79	± 1.98	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
<b>Viability (%)</b>	78.91	65.89	64.26	64.96	54.84	51.70	47.95	36.69
<b>SD</b>	± 0.00	± 0.34	± 1.31	± 0.30	± 0.49	± 1.12	± 4.54	± 4.09



Resuspended human hepatocytes suspension (0,5 \* 10<sup>6</sup> 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	24.58 ± 2.80
CYP2B6	4.64 ± 0.21
CYP3A4/5	9.72 ± 0.63

Cryopreserved human hepatocytes in suspension culture (0.5 \* 10<sup>6</sup> 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



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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			29/11/23

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