Updated on: 29th November 2023

# CERTIFICATE OF ANALYSIS

**Lot#:** CHM2305-HE-Z

## PRODUCT DESCRIPTION

**Reference:** HuHECSM-3D/4+
**Product:** Cryopreserved Human Hepatocytes
**Category:** Suspension, Metabolism certified
**Spheroid qualified:** Yes
*(see details below: 3D Spheroid formation section)*

**Isolation date:** 26th April 2023
**Initial Isolation Viability:** 76.00%
**Storage conditions**: -196°C using $LN_2$
**Sterility test:** negative for bacteria, yeast, and fungi

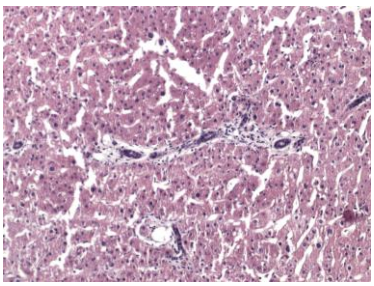
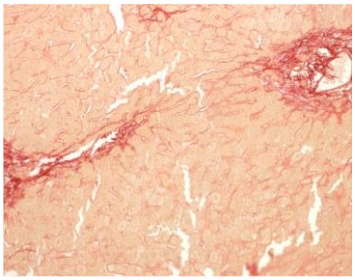
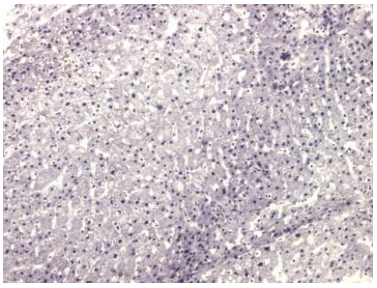
## DONOR DEMOGRAPHICS

| Species | Gender | Race | Age | BMI | Smoker | Alcohol Use | Drug Use |
|---|---|---|---|---|---|---|---|
| Human | Male | Caucasian | 76 | 24.91 | No | No | No |

| Pathology | Serological Data[1] |
|---|---|
| Cholangiocarcinoma | Tested negative less than 3 months before surgery |

Patient informed consent was obtained. [1]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 staining | Sirius Red staining | Oil red staining |
|---|---|---|
|  |  |  |

- Hematoxylin & Eosin: Parenchyma with very light microvesicular steatosis present and some detectable necrosis (anuclear hepatocytes) dispersed throughout the parenchyma. Cellular infiltrate in the portal triads close to bile ducts and portal vein

- Sirius red: Liver with light signs of fibrosis, with discrete accumulation of sirius red staining in portal areas and discrete bridging fibrosis. Also, observable matrix deposition in the sinusoidal vessels throughout the parenchyma

- Oil red: Very light vacuolation present in some hepatocytes and negative for oil red staining

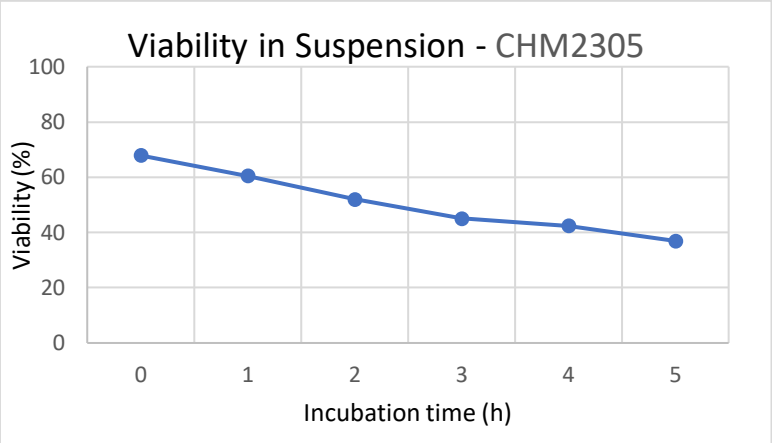
**Conclusions: Liver with complete washing at the sinusoidal level, with some areas exhibiting light matrix deposition. Most of the tissue devoid of vacuoles and/or fatty accumulation. Cellular infiltrate in the portal areas**

## CHARACTERIZATION FOR SUSPENSION CELLS

| Post Thaw Lot information | Result | SD | n |
|---|---|---|---|
| **Number of viable cells (cells/vial):** | $5.75x10^6$ | ± $1.05x10^6$ | 5 |
| **Post-thaw viability (%):** | 70.03 | ± 1.21 | 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 |
|---|---|---|---|---|---|---|---|---|
| **Viability (%)** | 67.86 | 58.79 | 60.44 | 52.76 | 52.01 | 45.04 | 42.36 | 36.94 |
| **SD** | ± 0.00 | ± 5.11 | ± 0.44 | ± 0.48 | ± 2.26 | ± 1.66 | ± 2.08 | ± 2.34 |




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**

Primary human hepatocytes self-assembled into a spheroid containing 5000 cells after 5 days in culture. These hepatic spheroids were cultured for 7-15 days in ultra-low attachment (ULA) plates with our 3D Culture Maintenance Media for hepatocytes (MHM3D). *For more information/protocols about 3D hepatocyte spheroids, contact us.*

## 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 | 15.26 ± 0.03 |
| CYP2B6 | 4.40 ± 0.47 |
| CYP3A4/5 | 7.25 ± 0.15 |

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 | Tittle | Signature | Cytes Biotechnologies, S.L. | Date |
|---|---|---|---|---|
| Pilar Sainz de la Maza | Quality Manager |  |  | 29/11/23 |