CYTES BIOTECHNOLOGIES S.L.

Updated on: 6th April 2022

# CERTIFICATE OF ANALYSIS

**Lot#:** CHF2204-HE-Z

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

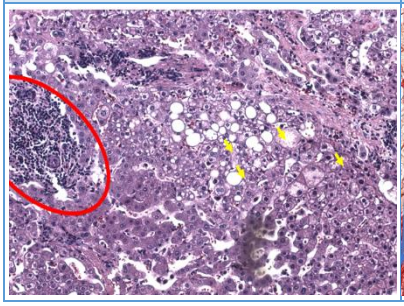
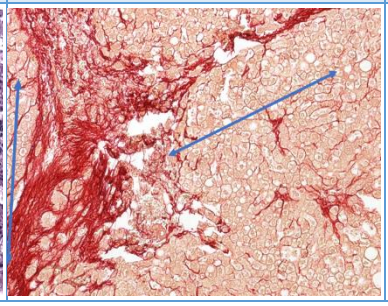
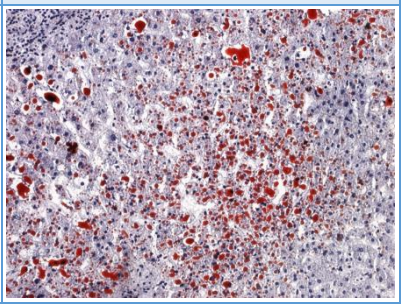
## DONOR DEMOGRAPHICS

| 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 | Tested negative less than 3 months before surgery |

Patient informed consent was obtained. 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. [1] **For donor's medication information, please contact us.*

## DONOR HISTOLOGY

| Hematoxylin & Eosin staining | Sirius Red staining | Oil red staining |
|---|---|---|
|  |  |  |

- 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).

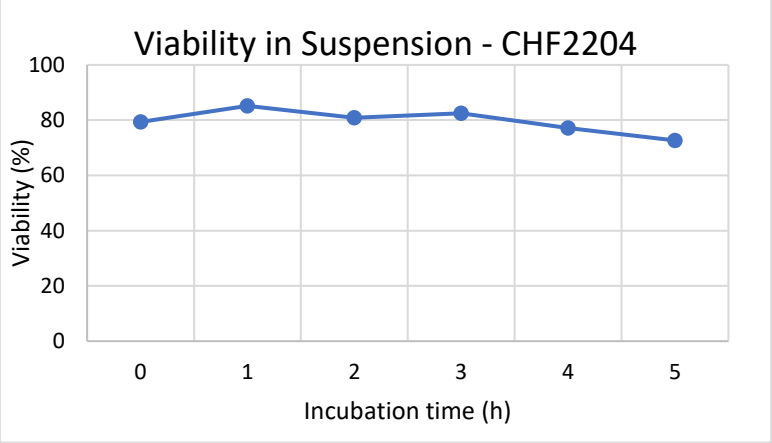
**Conclusions: Evident and extensive bridging fibrosis between portal triads. Widespread lipid accumulation in most of the hepatocytes with visible hepatic necrosis. Zones of granuloma tissue compatible with liver inflammation (NASH).**

## CHARACTERIZATION FOR SUSPENSION CELLS

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.

| Post Thaw Lot information | Result | SD | n |
|---|---|---|---|
| **Number of viable cells/vial:** | $3.06x10^6$ | ± $0.10x10^6$ | 3 |
| **Viability (%):** | 78.68 | ± 1.85 | 3 |

| 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 * $10^6$ 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. |

## 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 | 12.39 ± 0.41 |
| CYP2B6 | 1.70 ± 0.09 |
| CYP3A4/5 | 3.49 ± 0.05 |

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 |  | | 06/04/22 |