CYTES BIOTECHNOLOGIES S.L.

Updated on: 14th June 2023

# CERTIFICATE OF ANALISYS

**Lot#:** CHF2217-KC-Z

## PRODUCT DESCRIPTION

**Reference:** HuKC
**Product:** Cryopreserved Human Kupffer Cells
**Cellular passage:** P0
**Size/Quantity:** 500.000 cells/vial

**Isolation date:** 4th July 2022
**Storage conditions**: -196°C using $LN_2$
**Sterility test:** Negative for bacteria, yeast, and fungi

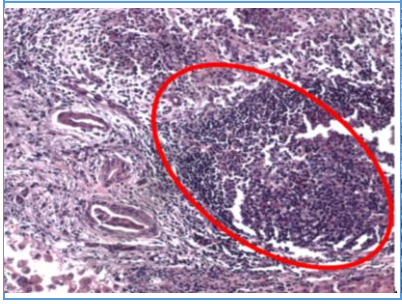
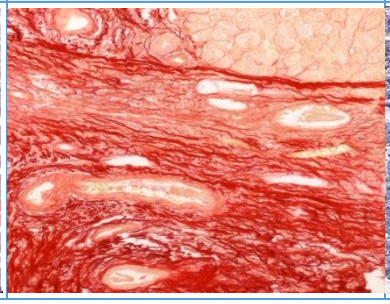
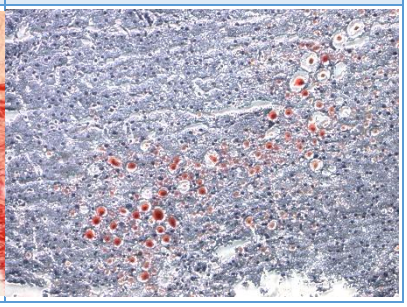
## DONOR DEMOGRAPHICS

| Species | Gender | Race | Age | BMI | Smoker | Alcohol Use | Drug Use |
|---|---|---|---|---|---|---|---|
| Human | 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. [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: 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.

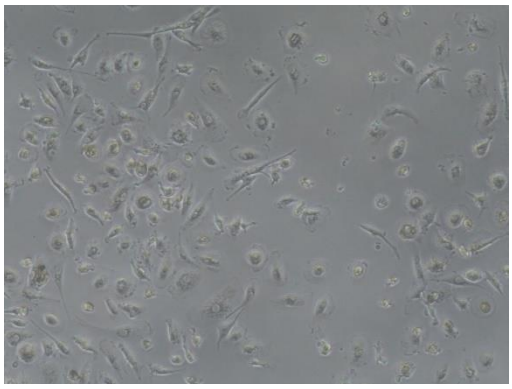
- Oil red: Small areas with "fatty" hepatocytes, but with hepatocyte ballooning present, showing areas of necrosis and absence of parenchymal cells.

**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.**

## CHARACTERIZATION FOR KUPFFER CELLS

| Post Thaw Lot information | |
|---|---|
| **Number of viable cells/vial:** | $5.84x10^5 \pm 0.23x10^5$ |
| **Cell seeding density (cells/cm²):** | 100.000-150.000 |

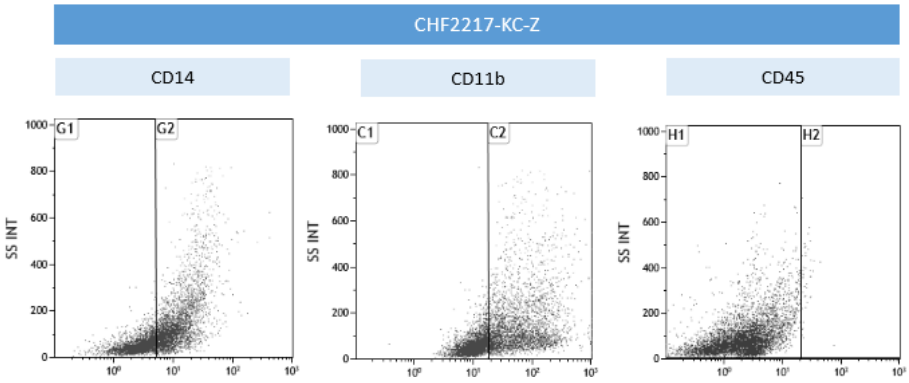
**Cell morphology 6 days**



Kupffer cells were thawed and seeded according to Cytes Biotechnologies protocol. The yield and viability post-thawing was assessed by using the trypan blue exclusion assay. Resuspended human Kupffer cells from post-thaw assessment were plated in 48-well plates in Kupffer Maintenance Medium. Kupffer cells will begin to attach at approximately day 6, at with point the medium can be replaced.

## FLOW CYTOMETRY ANALYSIS

Human Kupffer cells cultured for 6 days were analyzed by flow cytometry using specific cell markers.



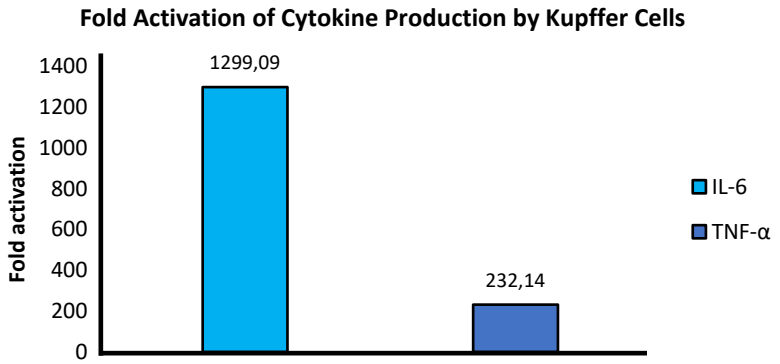

**Flow cytometry of human Kupffer cells**. Representative dot-plots of the markers used to characterize human Kupffer cells.

| Cell marker | Positive cells |
|---|---|
| **CD14** | 54.67% |
| **CD11b** | 48.9% |
| **CD45** | N/A |

**Flow cytometry analysis from human Kupffer cell population**. Cryopreserved human Kupffer cells were thawed and plated in 48-well plates in Kupffer Culture Medium. After 6 days in culture and without any medium replacement, cells were detached and stained with CD14, CD11b, and CD45. Results were expressed in terms of marker expression *vs*. control (%). Data were obtained by using a Beckman Coulter Gallios Flow Cytometer and were analyzed by using Kaluza Analysis 2.1 software.

## LPS STIMULATION

Human Kupffer cells cultured for 6 days and were treated with LPS. Fold activation of IL-6 and TNF-α are listed in the graph below.




**Fold activation of cytokine production by Kupffer cells.** Cryopreserved human Kupffer cells were thawed and plated in 48-well plates in Kupffer Culture Medium. After 6 days in culture and without any medium replacement, cells were treated with 1 ug/ml LPS for 4 hours. At the end of the treatment period, RNA was isolated for mRNA analysis. Results were expressed in terms of fold activation of IL-6 and TNF- α. Data were obtained by using a QuantStudio™ 5 Real-Time PCR System.

**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 human stellate cells 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 | | | 14/06/23 |