CYTES BIOTECHNOLOGIES S.L.

Updated on: 14th June 2023

CERTIFICATE OF ANALISYS

Lot#: CHM2215-KC-Z

PRODUCT DESCRIPTION

Reference: HuKC
Product: Cryopreserved Human Kupffer Cells
Cellular passage: P0
Size/Quantity: 1.000.000 cells/vial

Isolation date: 13th June 2022
Storage conditions: -196°C using LN_2
Sterility test: Negative for bacteria, yeast, and fungi

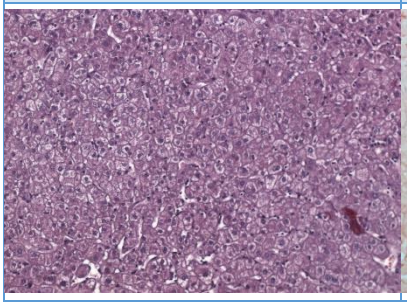
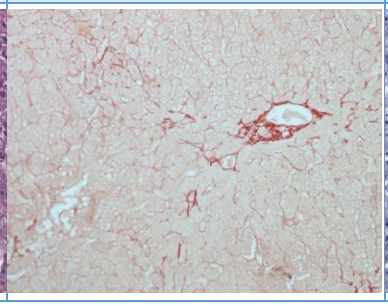
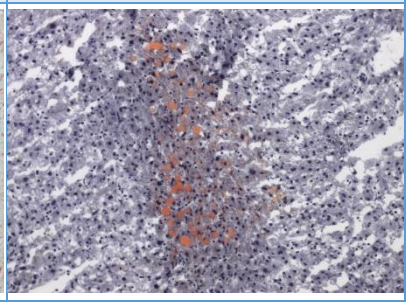
DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	69	27.18	No	No	No

Pathology	Serological Data[1]
Metastatic tumor	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: 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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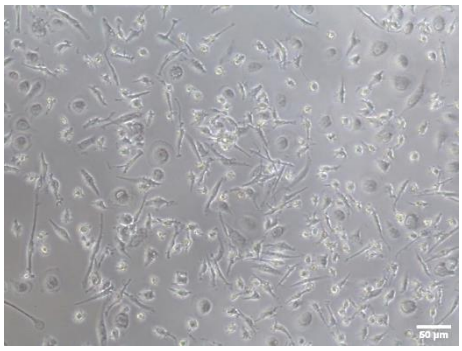
CHARACTERIZATION FOR KUPFFER CELLS

Post Thaw Lot information	
Number of viable cells/vial:	$1.18x10^6 \pm 0.84x10^6$
Viability (%):	91.37 ± 2.62
Cell seeding density (cells/cm²):	100.000-150.000

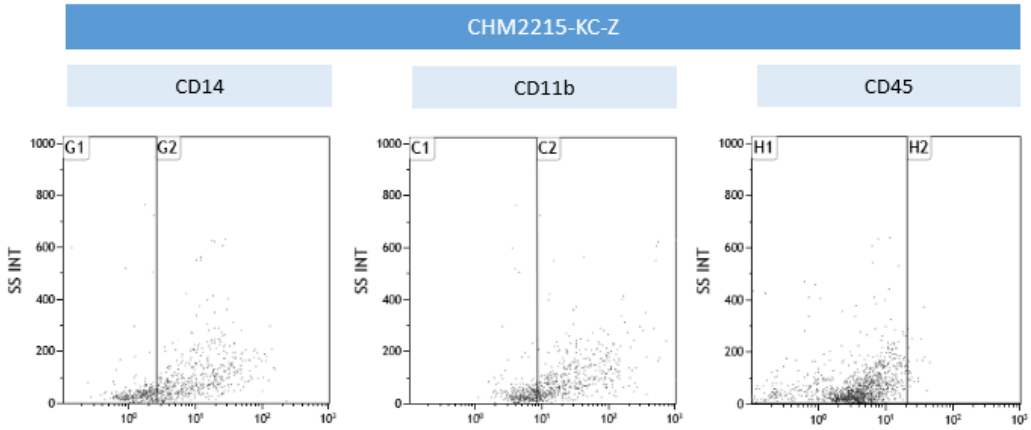
Cell morphology 6 days



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 Culture Kupffer 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.



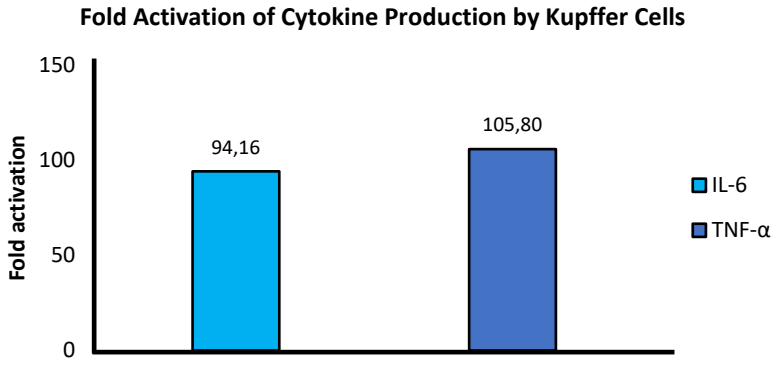
Flow cytometry of human Kupffer cells. Representative dot-plots of the markers used to characterize human Kupffer cells.

Cell marker	Positive cells
CD14	61.92%
CD11b	58.18%
CD45	N/A

Flow cytometry analysis from human Kupffer cell population. Cryopreserved human Kupffer cells were thawed and plated in 48-well plates in Culture Kupffer 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 were cultured for 6 days and then, 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

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