

## CERTIFICATE OF ANALISYS

Lot#: CHF2217-SC-P2-Z

### PRODUCT DESCRIPTION

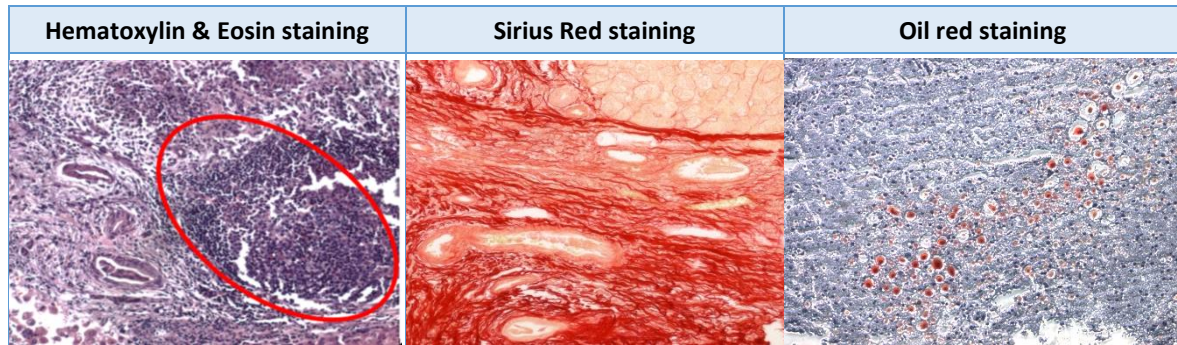
**Reference:** HuSC**Product:** Cryopreserved Human Stellate Cells**Cellular passage:** P2**Size/Quantity:** 100.000 cells**Isolation date:** 4<sup>th</sup> July 2022**Storage conditions:** -196°C using LN<sub>2</sub>**Sterility test:** Negative for mycoplasma, bacteria, yeast, and fungi

### DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	76	32.81	No	No	No
Pathology			Serological Data <sup>1</sup>				
Hepatocellular Carcinoma, NASH diagnosis			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, Hepatitis 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: 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.

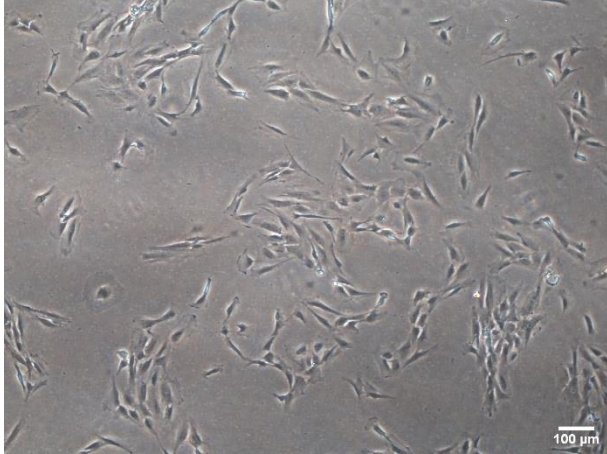
- Sirius red: Liver with noticeable fibrosis with fibrotic bridges between periportal areas, showing septal formation between acinar units. Little matrix deposition in the sinusoidal areas and increased deposition in pericentral areas.

- 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.

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## CHARACTERIZATION FOR HUMAN STELLATE CELLS

Post-thawing data	
Number of viable cells/vial:	> 100.000
Cell seeding density (cells/cm <sup>2</sup> ):	5.000
Cell morphology	
	

Human stellate cells were thawed and seeded according to BeCytes Biotechnologies protocol. The number of cells and viability post-thawing was assessed by using the trypan blue exclusion assay. Phase-contrast image is shown on the panel.

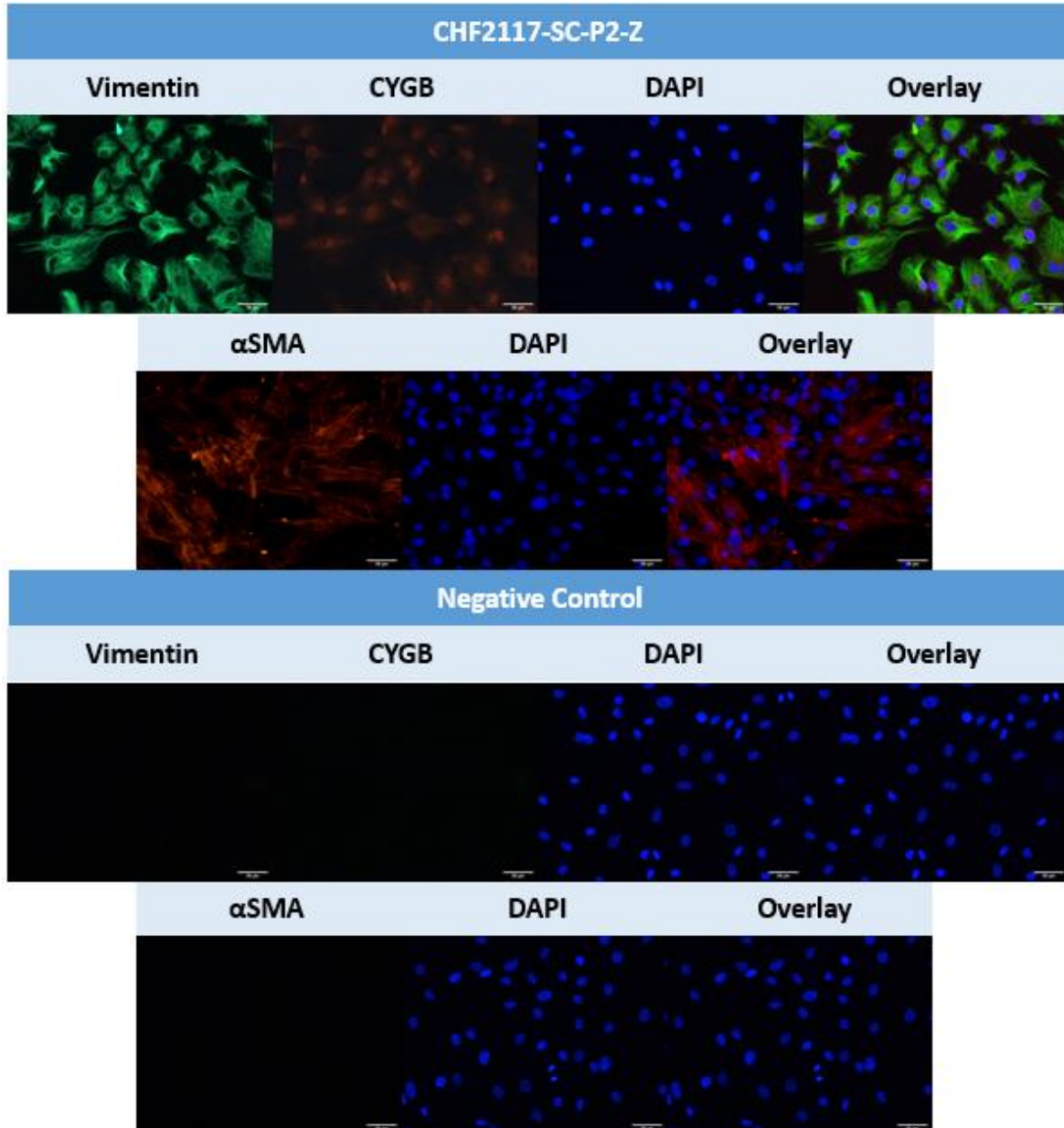
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**CYTES BIOTECHNOLOGIES, SL.**

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## IMMUNOFLUORESCENCE ANALYSIS

Human stellate cells are positive for vimentin, and cygb and negative for  $\alpha$ -smooth muscle actin when they are quiescent. When they become activated, they start to express  $\alpha$ -smooth muscle actin.



Cells were cultured on 8 well chamber slide till reach the confluence. The first panel shows green immunofluorescence for vimentin which is evident in the cell body and cytoplasmic processes in the cultured stellate cells. Red immunofluorescence for cygb is also positive. The second panel shows negative expression for  $\alpha$ -smooth muscle actin. Both panels show blue immunofluorescence for DAPI, a cellular nuclei marker. Negative controls are showed on the bottom of each panel with all the markers used for the SC.

**If you need help for an experiment, just contact us, our experts will be pleased to assist you**



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**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	Title	Signature	Cytes Biotechnologies, S.L.	Date
Pilar Sainz de la Maza	Quality Manager			03/04/23

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