

## CERTIFICATE OF ANALISYS

Lot#: CHM2104-SC-P3-Z

### PRODUCT DESCRIPTION

**Reference:** HuSC

**Product:** Cryopreserved Human Stellate Cells

**Cellular passage:** P3

**Size/Quantity:** 250.000 cells/vial

**Isolation date:** 25<sup>th</sup> May 2021

**Storage conditions:** -196°C using LN<sub>2</sub>

**Sterility test:** Negative for mycoplasma, bacteria, yeast, and fungi

### DONOR DEMOGRAPHICS

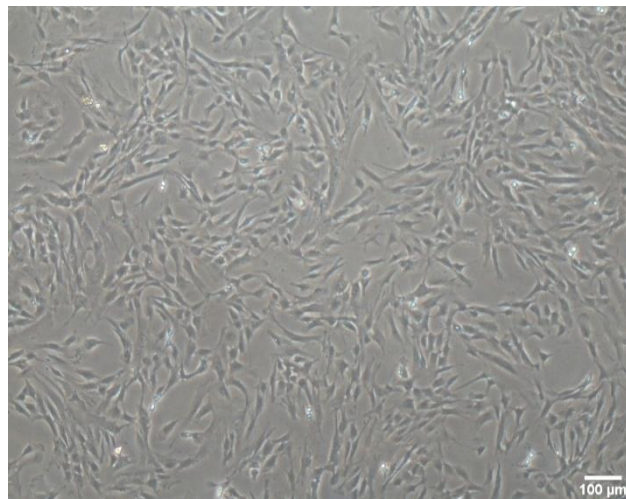
Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	68	25.06	No	No	No
Pathology		Serological Data <sup>1</sup>					
Hepatic metastases		Tested negative less than 3 months before surgery					

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.*

### CHARACTERIZATION FOR HUMAN STELLATE CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells/vial:	4.10x10 <sup>5</sup>	± 0.09 x10 <sup>5</sup>	6
Viability (%):	88.71	± 3.51	6
Cell seeding density (cells/cm <sup>2</sup> ):	5.000-8.000		

#### Cell morphology



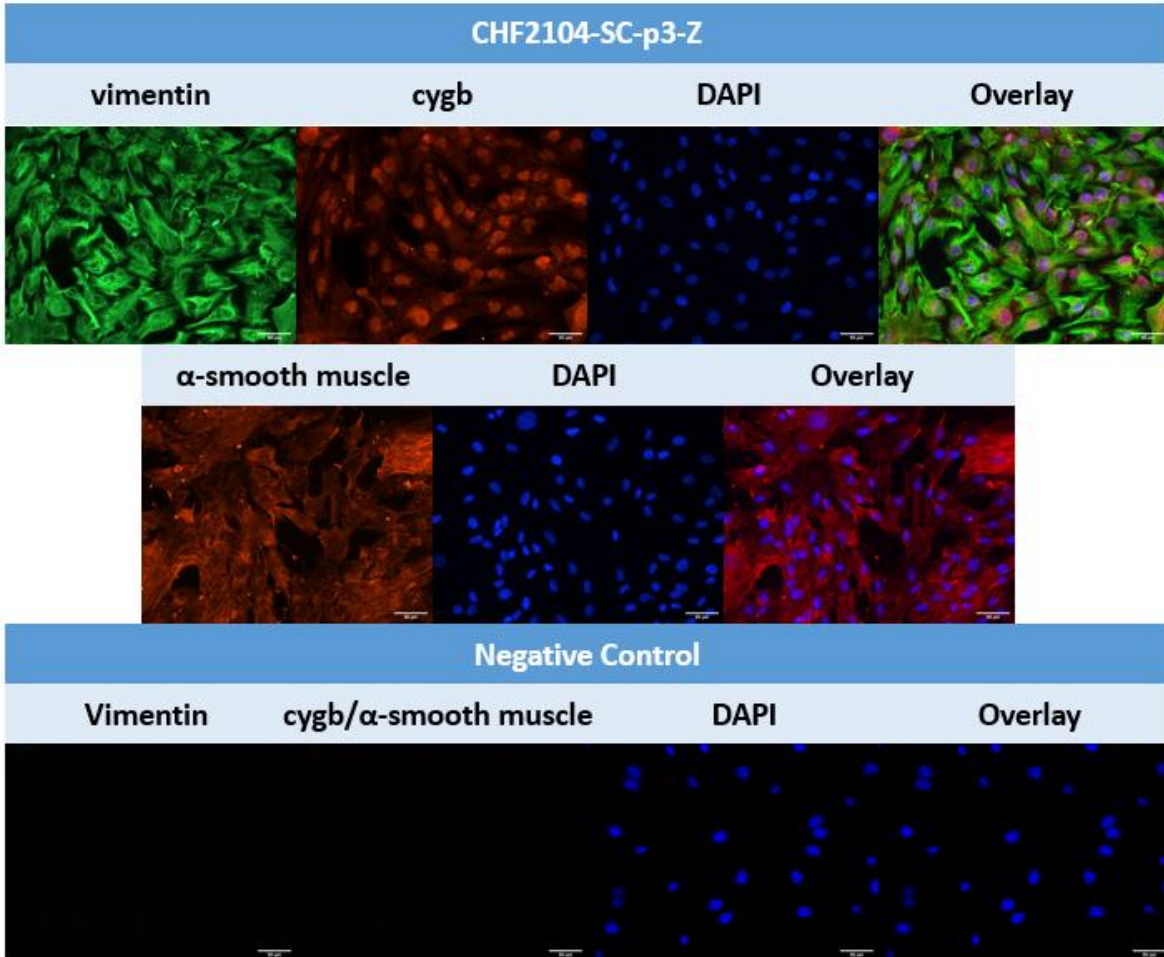
Human stellate cells were thawed and seeded according to BeCYtes Biotechnologies protocol. The number of cells and viability post-thawing was assessed by using the trypan blue exclusion assay. Phase-contrast image 5 days after seeding is shown on the panel.

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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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**CYTES BIOTECHNOLOGIES, SL.**

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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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