

CERTIFICATE OF ANALYSIS

Lot#: CHM2210-LEC/LSEC-P1-Z

PRODUCT DESCRIPTION

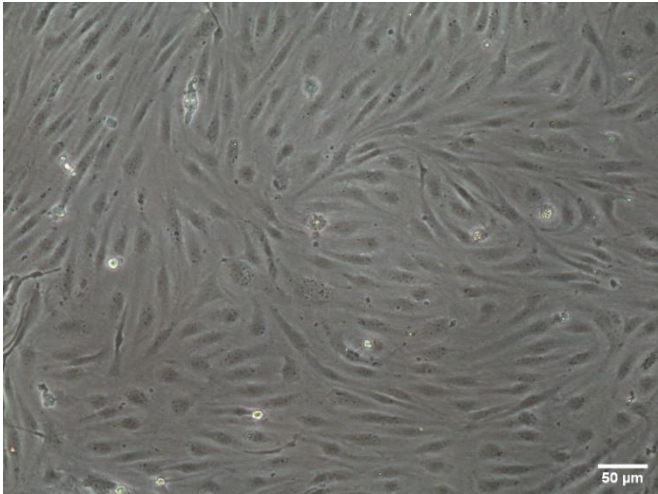
Reference: HuLEC/LSEC**Product:** Cryopreserved Human Liver Endothelial and Sinusoidal Endothelial cells**Cellular Passage:** P1**Size/Quantity:** >100.000 cells/vial**Isolation date:** 6th May 2022**Storage conditions:** -196°C**Sterility test:** negative for mycoplasma, bacteria, yeast, and fungi.

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	59	24.77	No	No	No
Pathology		Serological Data ¹					
Cholangiocarcinoma		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.

CHARACTERIZATION FOR HUMAN LIVER ENDOTHELIAL AND SINUSOIDAL ENDOTHELIAL CELLS

Post-thawing data	
Number of viable cells/vial:	> 100.000
Cell seeding density (cells/cm ²):	8.000-12.000
Cell morphology	
	

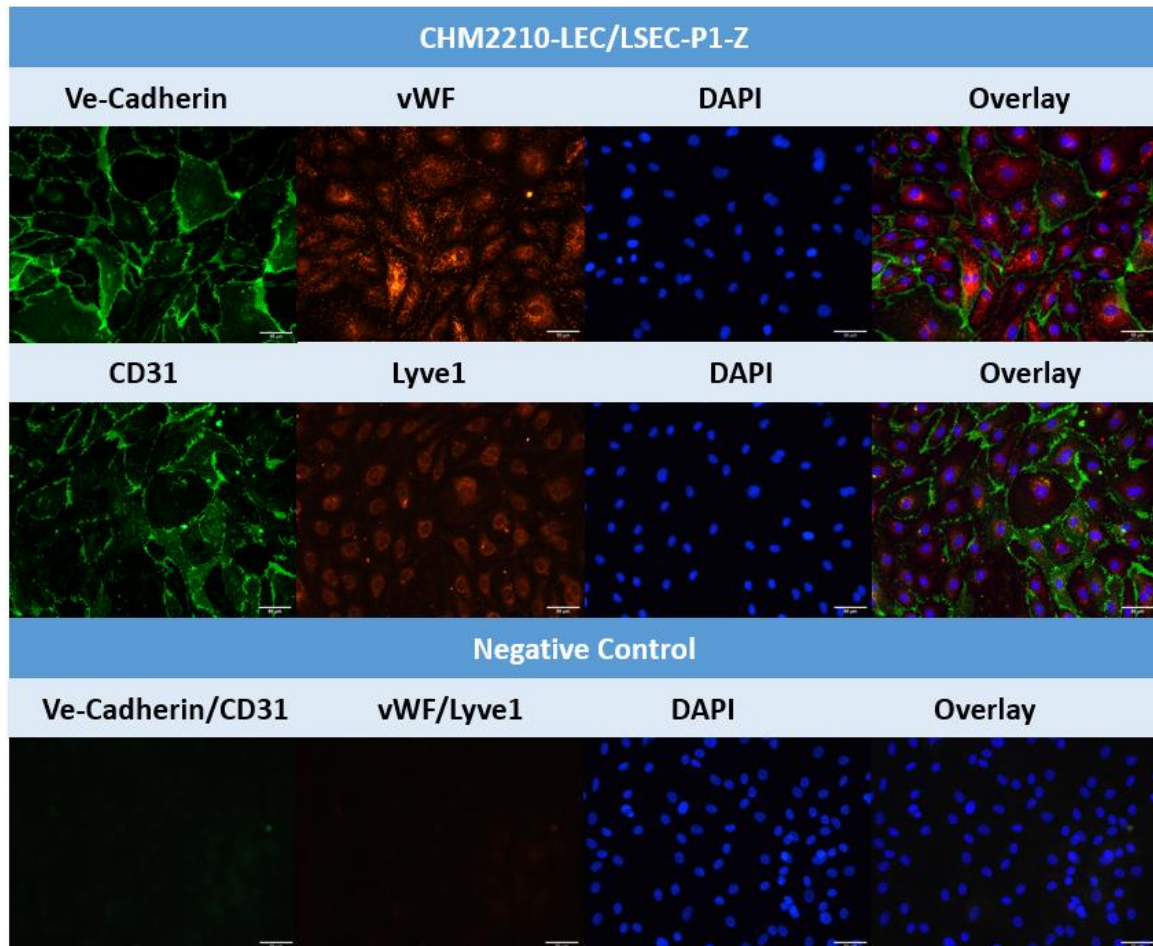
Human liver endothelial and sinusoidal endothelial cells were thawed and seeded according to Cytes Biotechnologies protocol. The number of cells and viability post-thawing was assessed by using the trypan blue exclusion assay. Phase-contrast image is shown on the panel.

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CHARACTERIZATION FOR HUMAN LIVER ENDOTHELIAL AND SINUSOIDAL ENDOTHELIAL CELLS

IMMUNOFLUORESCENCE ANALYSIS

The immunofluorescence was performed by selecting the specific markers Ve-cadherin, von Willebrand factor (vWF), and CD31 for liver endothelial cells. In this characterization, the marker Lyve-1, specific for liver sinusoidal endothelial cells has been also included to have an idea about the percentage of expression in the cells provided.





Cells were cultured on 8 well chamber slide coated with fibronectin till reach the confluence. Human liver endothelial and sinusoidal endothelial cells were fixed and stained with Ve-cadherin and von Willebrand factor, as well as CD31 and Lyve-1. In the first panel, green fluorescence for Ve-Cadherin is evident as membrane marker, as well as the expression of red fluorescence for the marker vWF. In the second panel, the membranal marker CD31 is clearly expressed with green fluorescence and the lyve-1 marker is expressed with red fluorescence. Both panels included a staining with DAPI, a blue fluorescence marker specific for the cellular nuclei. Negative controls are showed on the bottom of each panel with all the markers used for the LEC and LSEC.

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 primary human liver endothelial 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			08/07/22

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