

CERTIFICATE OF ANALYSIS

Lot#: CHF2110-HE-Z

PRODUCT DESCRIPTION

Reference: HuHECP/4-
Product: Cryopreserved Human Hepatocytes
Category: Plateable
Spheroid qualified: NO
(see details below: 3D Spheroid formation section)

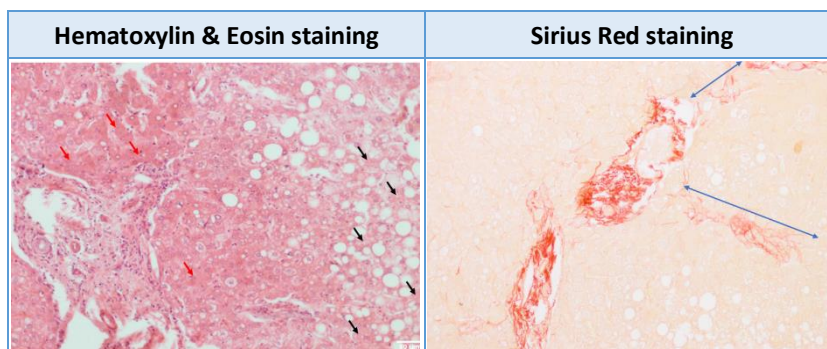
Isolation date: 21st September 2021
Initial Isolation Viability: 85.54%
Storage conditions: -196°C using LN₂
Sterility test: negative for bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Female	Caucasian	81	32.46	No	No	No
Pathology		Serological Data ¹					
Cholangiocarcinoma		Tested negative less than 3 months before surgery					

Patient informed consent was obtained. ¹The 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. ²The donor was tested negative for SARS-CoV-2 before surgery. *For donor's medication information, please contact us.*

DONOR HISTOLOGY



- Hematoxylin & Eosin: Large areas of the parenchyma with vacuolated hepatocytes and significant hepatocellular ballooning (Estimated hepatic steatosis >30%) and manifest centrilobular necrosis (black arrows). Evidence of hepatic proliferation in periportal areas (eosinophilic small hepatocytes) probably due to increased hepatocyte turnover (red arrows).

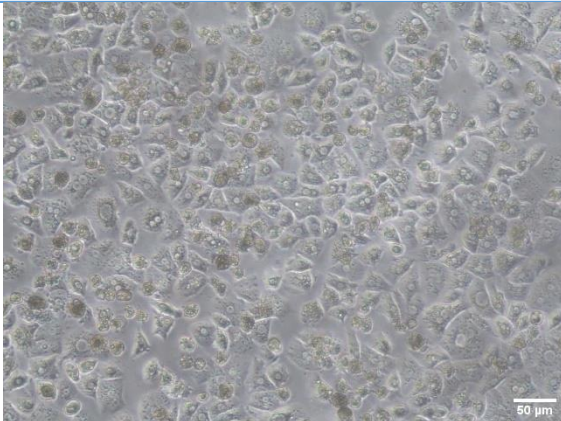
- Sirius red: Sirius Red staining showing a small increase in matrix deposition in and around portal triads and perisinusoidal/pericellular structures (red staining). There's no obvious formation of septa yet, but there is obvious deposition of collagen fibers between portal triads (blue arrows). By the mild fibrotic matrix deposition, this tissue is scored as a F1b/F2 in the NASH CRN score.

Conclusions: This is a mildly fibrotic tissue sample with extensive hepatic steatosis (>30% steatotic hepatocytes) and a high number of red blood cells in the vessels.

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CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells (cells/vial):	5.01x10 ⁶	± 2.10x10 ⁶	3
Post-thaw viability (%):	89.25	± 3.63	3
Days in culture after thaw (days):	2	± 0.00	1
MONOLAYER ASSESSMENT² Plateable: YES Confluence: 80%			
Seeding density in 24 well recommended: 2.37x10⁵ cells/cm²			
Cell morphology 24h			
			

Human hepatocytes were thawed and seeded according to Cytes Biotechnologies culture protocol. The yield and viability were determined by a trypan blue exclusion assay after the thawing process. ²Resuspended human hepatocytes from post-thaw assessment were plated in collagen-coated 24-well plates in hepatocyte plating medium. Cells were refreshed with hepatocytes maintenance medium at first medium during the first change of medium on the day of thawing. Maintenance medium was replaced in the culture every day. If images from the 96-well plates are needed, please contact us.



3D SPHEROID FORMATION

Cytes **does not guarantee** that these primary hepatocytes will be suitable for 3D culture and creation of spheroid structures while using Cytes protocols.

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 primary human hepatocytes 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			24/03/23

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CYTES BIOTECHNOLOGIES, S.L.

CELL COUNTING

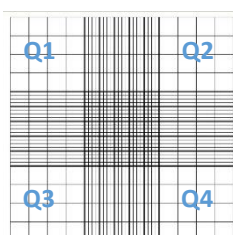
Lot #: _____

Date: ____/____/____

MORPHOLOGY

- | | | | |
|------------------------------------------|--------------------------------------------|-----------------------------------------|--------------------------------------------|
| <input type="checkbox"/> Clear cytoplasm | <input type="checkbox"/> Rounded shape | <input type="checkbox"/> Cell swelling | <input type="checkbox"/> Hardly any debris |
| <input type="checkbox"/> Clear membranes | <input type="checkbox"/> Membrane blebbing | <input type="checkbox"/> Lipid droplets | <input type="checkbox"/> Prevalent debris |

TRYPAN BLUE COUNTING RESULTS



NEUBAUER CHAMBER COUNTING					
Quadrant	Live cells	+	Dead cells	=	Total cells
Quadrant 1		+		=	
Quadrant 2		+		=	
Quadrant 3		+		=	
Quadrant 4		+		=	
Total		+		=	

VIABILITY

$$\frac{(\text{Live cells})}{(\text{Total cells})} \times 100 = \text{Viability (\%)}$$

YIELD

$$\frac{(\text{Total cells}) \times (\text{Dilution factor}) \times 10^4 \times (\text{Current volume}) \text{ ml}}{(\text{Counted quadrants})} = \text{cells (Total number of cells)}$$

**This factor (10⁴) is applicable when it is used a Hemocytometer*

SEEDING DENSITY

$$\frac{(\text{Desired number of cells})}{(\text{Total number of cells})} \times \frac{\text{cells} \times (\text{Current volume}) \text{ ml}}{\text{cells}} = \text{ml (Volume needed for your cells)}$$

Keep in mind the final volume per dish or plate to use (Volume needed) and then calculate the needed volume to add: $(\text{Total volume well}) \text{ ml} - (\text{Cells total volume}) \text{ ml} = \text{ml (Volume to add)}$

Surface of the most common plates for culture:

Brand	24-well plate	96-well plate
ThermoFisher	1.90 cm ² /well	0.32 cm ² /well
Corning®	2.00 cm ² /well	0.36 cm ² /well
Falcon®	1.90 cm ² /well	0.32 cm ² /well
Eppendorf	2.08 cm ² /well	0.37 cm ² /well

COMMENTS

COUNTED BY:

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