

Updated on: 28<sup>th</sup> October 2024

## CERTIFICATE OF ANALYSIS

Lot#: CHM2420-L-HE-Z

### PRODUCT DESCRIPTION

**Reference:** HuHECP/4+  
**Product:** Cryopreserved Human Hepatocytes  
**Category:** Plateable  
**Spheroid qualified:** Yes  
**Specific culture requirements:** No



**Isolation date:** 17<sup>th</sup> October 2024  
**Initial Isolation Viability:** 92.00%  
**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	54	23.5	N/A	N/A	N/A
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 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.*

### CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
<b>Number of viable cells (cells/vial):</b>	5.36x10 <sup>6</sup>	± 0.23x10 <sup>6</sup>	2
<b>Post-thaw viability (%):</b>	88.42	± 1.07	2
<b>Days in culture after thaw (24w):</b>	N/A	± 0.00	1
<b>Days in culture after thaw (96w):</b>	N/A	± 0.00	1
<b>MONOLAYER ASSESSMENT<sup>2</sup> Plateable: YES</b>		<b>Confluence 24h: 95%</b>	
<b>Seeding density in 24 well recommended:</b>		2.1x10 <sup>5</sup> cells/cm <sup>2</sup>	
<b>Seeding density in 96 well recommended:</b>		2.81x10 <sup>5</sup> cells/cm <sup>2</sup>	
Cell morphology 24h		Cell morphology 96h	
			

Human hepatocytes were thawed and seeded according to BeCytes Biotechnologies culture protocol. The yield and viability were determined by a trypan blue exclusion assay after the thawing process. <sup>2</sup>Resuspended human hepatocytes from post-thaw assessment were plated in collagen-coated 24-well plates in hepatocyte plating medium. Cells were refreshed with hepatocytes maintenance

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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





Primary human hepatocytes self-assembled into a spheroid containing 1000 cells after 4 days in culture. These hepatic spheroids were cultured for 7-15 days in ultra-low attachment (ULA) plates with our 3D Culture Maintenance Media for hepatocytes (MHM3D). *For more information/protocols about 3D hepatocyte spheroids, contact us.*

**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			28/10/2024

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### CELL COUNTING

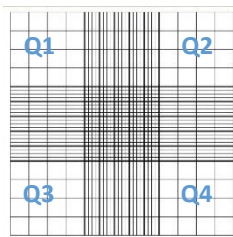
Lot #: \_\_\_\_\_

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

#### MORPHOLOGY

- Clear cytoplasm
- Rounded shape
- Cell swelling
- Hardly any debris
- Clear membranes
- Membrane blebbing
- Lipid droplets
- Prevalent debris

#### TRYPAN BLUE COUNTING RESULTS



NEUBAUER CHAMBER COUNTING					
Quadrant	Live cells	+	Dead cells	=	Total cells
Quadrant 1		+		=	
Quadrant 2		+		=	
Quadrant 3		+		=	
Quadrant 4		+		=	
<b>Total</b>		+		=	

#### 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 (10<sup>4</sup>) is applicable when it is used a Hemocytometer*

#### SEEDING DENSITY

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

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

#### Surface of the most common plates for culture:

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

#### COMMENTS

#### COUNTED BY:

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