

## CERTIFICATE OF ANALYSIS

Lot#: NHM2261-HE-N

### PRODUCT DESCRIPTION

**Reference:** HuHECP/4-  
**Product:** Cryopreserved Human Hepatocytes  
**Category:** Plateable, Cytochrome P450 inducible  
**Spheroid qualified:** NO  
*(see details below: 3D Spheroid formation section)*

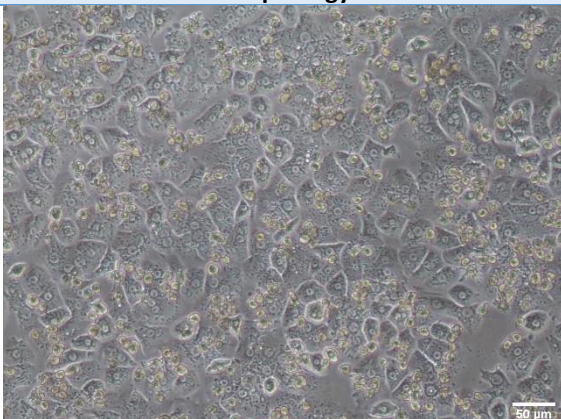
**Isolation date:** 5<sup>th</sup> July 2019  
**Storage conditions:** -196°C using LN<sub>2</sub>  
**Sterility test:** negative for mycoplasma, bacteria, yeast, and fungi

### DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	HLA	Smoker	Alcohol Use	Drug Use	COD
Human	Male	Caucasian	5	16.4	A23, A24, B8, B51, C07, C15	No	None	None	Anoxia/ Drowning

Patient informed consent was obtained. The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C, and syphilis.

### CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
<b>Number of viable cells (cells/vial):</b>	0.81x10 <sup>6</sup>	± 0.40x10 <sup>6</sup>	3
<b>Post-thaw viability (%):</b>	77.02	± 7.21	3
<b>Days in culture after thaw (24w):</b>	4	± 0.00	1
<b>MONOLAYER ASSESSMENT<sup>1</sup> Plateable: YES Confluence 24h: 90%</b>			
<b>Seeding density in 24 well recommended: 2.12x10<sup>5</sup> cells/cm<sup>2</sup></b>			
<b>Cell morphology 24h</b>			
			

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. <sup>1</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 medium at first medium during the first change of medium on the day of thawing. Maintenance medium was replaced in the culture every day.

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### 3D SPHEROID FORMATION



#### Spheroid morphology

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

**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	Tittle	Signature	Cytes Biotechnologies, S.L.	Date
Pilar Sainz de la Maza	Quality Manager			12/06/23

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**CYTES BIOTECHNOLOGIES, SL.**

Parc Científic de Barcelona, C/Baldiri Reixac 4-8 | [www.cytesbiotechnologies.com](http://www.cytesbiotechnologies.com) | [info@cytesbiotech.com](mailto:info@cytesbiotech.com) | P.+34 934034553



## CELL COUNTING

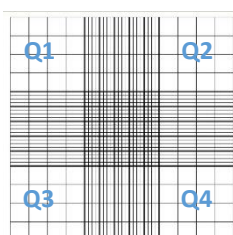
Lot #: \_\_\_\_\_

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

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

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

## SEEDING DENSITY

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

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

## Surface of the most common plates for culture:

Brand	24-well plate	96-well plate
ThermoFisher	1.90 cm <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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