

Updated on: 20<sup>th</sup> April 2021

## CERTIFICATE OF ANALYSIS

**Lot#:** Barcelona0002

### PRODUCT DESCRIPTION

**Reference:** HuHeCS/4+

**Product:** Human Hepatocytes

**Category:** Cryopreserved Suspension

**Isolation date:** 14<sup>th</sup> September 2015

**Initial Isolation Viability:** 88%

**Storage conditions:** -196°C

### DONOR DEMOGRAPHICS

Species	Sex	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	75	30	No	No	No

Pathology	Medications	Serological Data
Adenocarcinoma hepatic metastases	Omeprazol, antihypertensives, adiro, levodopa	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. Donor medical history was also examined prior to accepting this donor. The donor was tested negative for SARS-CoV-2 before surgery.

For *in vitro* use only, not to be used for clinical application. Products distributed by Cytes Biotechnologies may contain human material that should be treated as potentially hazardous.



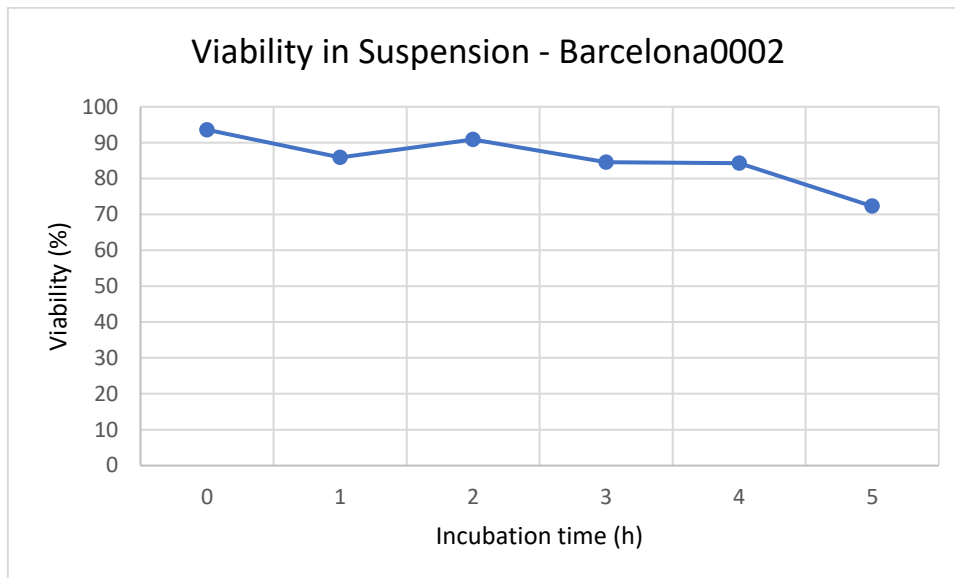
**CHARACTERIZATION FOR PLATEABLE CELLS**

**Lot#:** Barcelona0002

Post Thaw Lot information			
	Result	SD	n
<b>Number of viable cells (cells/vial):</b>	5.62x10 <sup>6</sup>	± 1.25	3
<b>Post-thaw viability (%):</b>	93.72	± 0.25	3

Human hepatocytes were thawed according to Cytes Biotechnologies protocol. The post-thawing yield and viability (trypan blue exclusion assay) of hepatocytes were assessed after a purification process.

Time (h)	0	0.5	1	1.5	2	3	4	5
<b>Viability (%)</b>	93.59	89.42	85.90	86.60	90.90	84.50	84.26	72.28
<b>SD</b>	± 0.13	± 2.29	± 0.76	± 0.40	± 1.39	± 2.88	± 0.68	± 1.03



Resuspended human hepatocytes suspension (0,5 \* 10<sup>6</sup> cells in 0.5 ml medium) from the post-thaw assessment were incubated up to 5 h at 37°C. The assay was performed in 2 ml round-bottom tubes under shaking conditions (1000 rpm) using Eppendorf Thermomixer C. In the first two hours, samples were taken at every 30 min, after 2 h samples were taken at every 60 min. At each time point the viability of cells was calculated.

**CERTIFICATION:**

Name	Title	Signature	Date
Aina Soria	Quality Manager		20/04/21

Cytes Biotechnologies, S.L.



**CYTES BIOTECHNOLOGIES, SL.**

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## CELL COUNTING

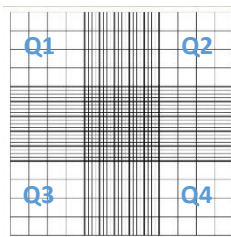
Lot #: \_\_\_\_\_

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		+		=	
<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

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

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 <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: