CYTES BIOTECHNOLOGIES S.L.

Updated on: 11th July 2023

CERTIFICATE OF ANALYSIS

Lot#: NHM2354-HE-N

PRODUCT DESCRIPTION

Reference: HuHECP/4-
Product: Cryopreserved Human Hepatocytes
Category: Plateable
Spheroid qualified: YES
(see details below: 3D Spheroid formation section)

Isolation date: 25th January 2023
Storage conditions: -196°C using LN_2
Sterility test: negative for mycoplasma, bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	Smoker	Alcohol Use	Drug Use	COD
Human	Male	Caucasian	2	No	No	No	Cardiac Arrest

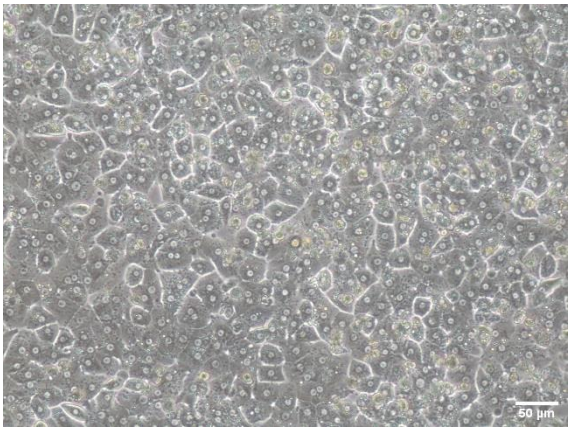
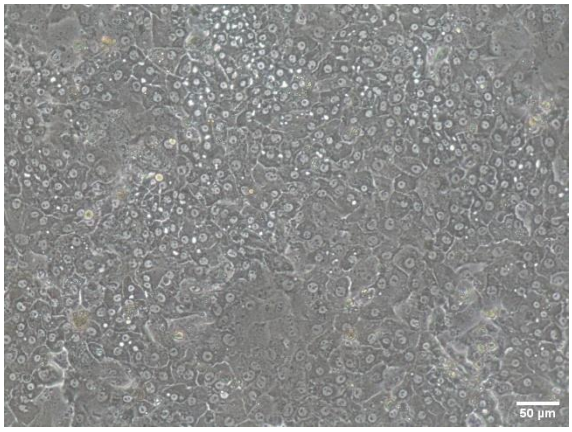
Patient informed consent was obtained. The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C, and syphilis.

CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	n
Number of viable cells (cells/vial):	$3.87x10^6$	± $0.37x10^6$	3
Post-thaw viability (%):	78.19	± 4.33	3
Days in culture after thaw (24w):	5	± 0.00	1

MONOLAYER ASSESSMENT[1] **Plateable: YES** **Confluence 24h:** 95%

Seeding density in 24 well recommended: $2.33x10^5$ cells/cm^2

Cell morphology 24h	Cell morphology 72h
	

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. [1]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.

TO OBTAIN THE RESULTS DESCRIBED ABOVE, PLEASE FOLLOW THE NO SPIN PROTOCOL PROVIDED BY BECYTES

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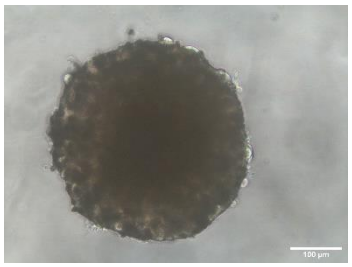
3D SPHEROID FORMATION

Spheroid morphology



Primary human hepatocytes self-assembled into a spheroid containing 5000 cells after 5 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	Tittle	Signature	Cytes Biotechnologies, S.L.	Date
Pilar Sainz de la Maza	Quality Manager			11/07/23

For basic research use only, not to be used for clinical or diagnostic applications. Products distributed by Cytes Biotechnologies may contain human material that should be treated as potentially biohazardous.

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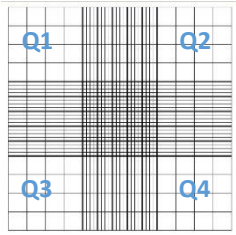
CELL COUNTING

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		+		=
Total		+		=

VIABILITY

$$\frac{(Live\ cells)}{(Total\ cells)} x100 = \qquad Viability\ (\%)$$

YIELD

$$\frac{(Total\ cells) \quad x\ (Dilution\ factor) \quad x\ 10^4 * x\ (Current\ volume) \quad ml}{(Counted\ quadrants)} = \qquad cells\ (Total\ number\ of\ cells)$$

**This factor (10^4) is applicable when it is used a Hemocytometer*

SEEDING DENSITY

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

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

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: