Updated on: 19th October 2023

CERTIFICATE OF ANALYSIS

Lot#: NHF2351-HE-N

PRODUCT DESCRIPTION

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

Isolation date: 9th September 2022
Storage conditions: -196°C using LN_2
Sterility test: negative for mycoplasma, bacteria, yeast, and fungi

DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	HLA	Smoker	Alcohol Use	Drug Use	COD
Human	Female	Caucasian	61	25	A02, A11 B13, B35, C04, C06	Yes	Yes	N/A	Diabetes Insipidus

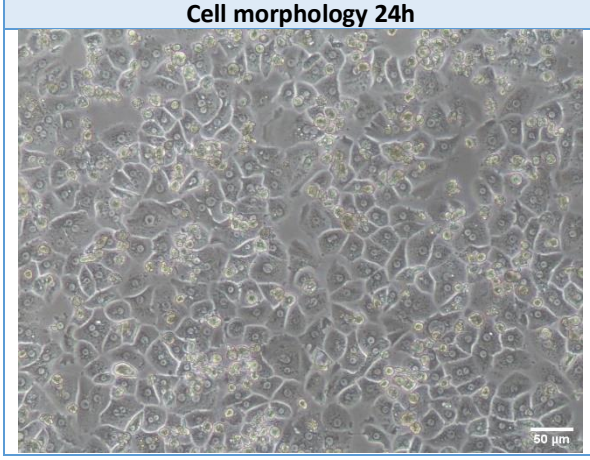
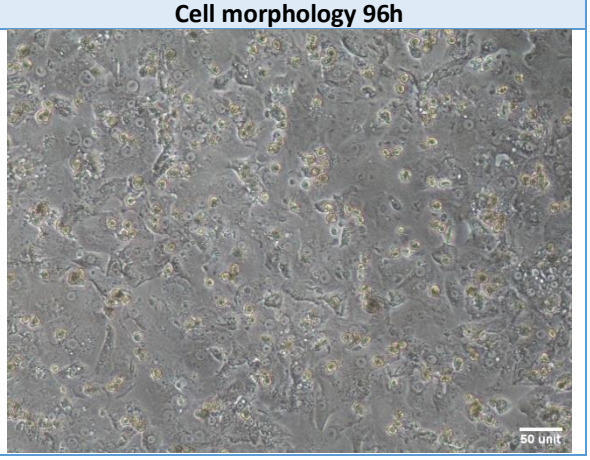
Patient informed consent was obtained. The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C, and syphilis.

CHARACTERIZATION FOR PLATEABLE CELLS

Post Thaw Lot information	Result	SD	N
Number of viable cells (cells/vial):	$2.99x10^6$	± $0.39x10^6$	2
Post-thaw viability (%):	78.48	± 3.84	2
Days in culture after thaw (24w):	4	± 0.00	1

MONOLAYER ASSESSMENT[1] **Plateable: YES** **Confluence 24h:** 75%

Seeding density in 24 well recommended: $2.10x10^5$ cells/cm^2

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. [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 during the first change of medium on the day of thawing. Maintenance medium was replaced in the culture every day.

3D SPHEROID FORMATION

Spheroid morphology
BeCytes **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			19/10/23

BeCYTES BIOTECHNOLOGIES

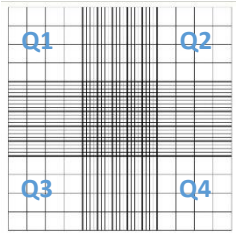
CELL COUNTING

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

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

YIELD

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

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

SEEDING DENSITY

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

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: