

CERTIFICATE OF ANALYSIS

Lot#: NHM2252-HE-N

PRODUCT DESCRIPTION

Reference: HuHECPMI/4+**Product:** Cryopreserved Human Hepatocytes**Category:** Plateable, Cytochrome P450 inducible**Spheroid qualified:** YES*(see details below: 3D Spheroid formation section)***Isolation date:** 6th May 2019**Storage conditions:** -196°C using LN₂**Sterility test:** negative for mycoplasma, bacteria, yeast, and fungi

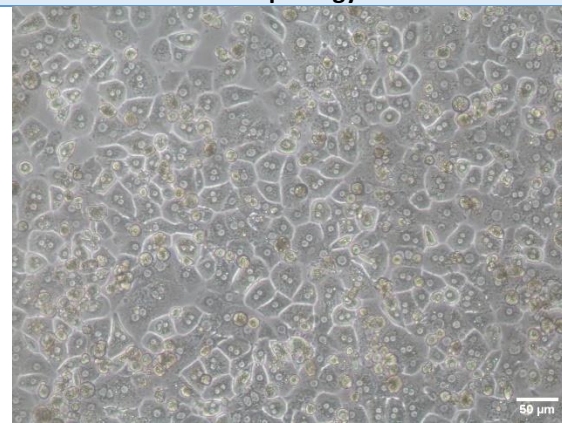
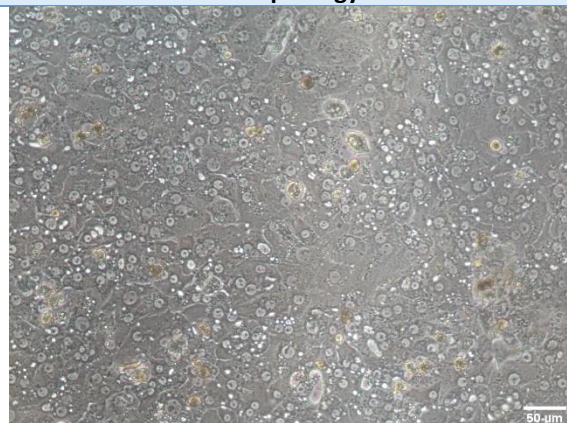
DONOR DEMOGRAPHICS

Species	Gender	Race	Age	BMI	HLA	Smoker	Alcohol Use	Drug Use	COD
Human	Male	Caucasian	45	29.1	A11, A68, B35, B53, C04, C04	No	1 drink 3-4x per year	N/A	Anoxia

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):	5.83x10 ⁶	± 1.00x10 ⁶	4
Post-thaw viability (%):	87.38	± 6.44	4
Days in culture after thaw (24w):	9	± 4.24	2
Days in culture after thaw (96w):	4	± 2	2

MONOLAYER ASSESSMENT¹ Plateable: YES**Confluence 24h: 90%****Seeding density in 24 well recommended:**2.12x10⁵ cells/cm²**Seeding density in 96 well recommended:**2.20x10⁵ cells/cm²**Cell morphology 24h****Cell morphology 96h**

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. ¹Resuspended 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. If images from the 96-well plates are needed, please contact us.

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3D SPHEROID FORMATION



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.*

INDUCTION FOR PLATEABLE CELLS

PHASE I: CYP ACTIVITIES EXPRESSED IN pmol/min/mg protein (mean ± SD)

Induction (Specific Activity)				
Enzyme	Basal Activity on day 1	Basal Activity on day 4	Induced Activity on day 4	n-Fold induction
CYP1A2	1.12 ± 0.09	1.28 ± 0.18	11.56 ± 0.64	9.01
CYP2B6	1.29 ± 0.30	0.18 ± 0.02	3.36 ± 0.14	18.72
CYP3A4	1.20 ± 0.20	1.95 ± 0.29	13.18 ± 0.33	6.78

Cryopreserved human hepatocytes were thawed and plated in 24well collagen I coated plates. Cells were overlaid with Matrigel® (Corning) in Human Hepatocyte Maintenance Medium at first medium change at day of thawing. Treatment (n=2 per compound) with vehicle control [0.15% (v/v) DMSO] or inducers (Rifampicin, β-Naphthoflavone and Phenobarbital) began 1-day post-plating and continued for 72 hours. At the end of induction, monolayers were rinsed with PBS and incubated with probe substrate solutions in culture media. See Table 1 for information on each probe substrate. Metabolites were quantified by LC-MS and normalized to protein content. The fold induction was calculated by dividing the induced activity by the vehicle basal activity on the same day in culture.

PHASE I: CYP450 mRNA induction

CYP (mRNA)	n-Fold Induction
CYP1A2	9 ± 1
CYP2B6	24 ± 4
CYP3A4	12 ± 4

Cryopreserved human hepatocytes were thawed, plated in 24well collagen I coated plates in Hepatocyte Plating Medium. Cells were overlaid with Matrigel® (Corning) in Human Hepatocyte Maintenance Medium at first medium change at day of thawing. Maintenance medium was replaced in the cultures daily. Treatment (n=2 per compound) with vehicle control [0.15% (v/v) DMSO] or inducers (Rifampicin, β-Naphthoflavone and Phenobarbital) began 1-day post-plating and continued for 72 hours. At the end of the treatment period, RNA was isolated for mRNA analysis.

Table 1. Substrates Phase I

Enzyme	Probe Substrate	Concentration (µM)	Incubation Time (min)	Metabolite
CYP1A2	Phenacetin	100	30	Acetaminophen
CYP2B6	Bupropion	500	30	Hydroxybupropion
CYP3A4	Midazolam	30	30	1-Hydroxymidazolam

PHASE II: UGTs & SULT ACTIVITIES 24h AFTER SEEDING EXPRESSED IN pmol/min/mg PROTEIN (mean ± SD)

Enzyme	Conjugate	pmol/min/mg
UGT	7-OH coumarin glucuronide	108.52 ± 16.07
SULT	7-OH coumarin sulfate	45.93 ± 3.91

Cryopreserved human hepatocytes were thawed, plated in 24well collagen I coated plates in Hepatocyte Plating Medium. Cells were overlaid with Matrigel® (Corning) in Human Hepatocyte Maintenance Medium at first medium change at day of thawing. On day 1, hepatocytes were incubated with 7-Hydroxycoumarin to assay for UDP-Glucuronosyltransferase (UGT) and Sulfotransferase (SULT) activities. See Table 2 for information on each probe substrate. Metabolites were quantified by LC-MS and normalized to protein content.

Table 2. Substrates Phase II



Enzyme	Probe Substrate	Concentration (µM)	Incubation Time (min)	Metabolite
UGT	7-Hydroxycoumarin	100	30	7-Hydroxycoumarin-glucuronide
SULT	7-Hydroxycoumarin	100	30	7-Hydroxycoumarin-sulfate

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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			10/04/23

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CELL COUNTING

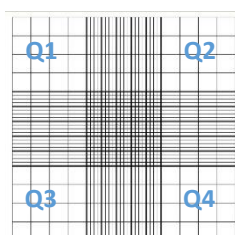
Lot #: _____

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		+		=	
Total		+		=	

VIABILITY

$$\frac{\text{(Live cells)}}{\text{(Total cells)}} \times 100 = \text{Viability (\%)}$$

YIELD

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

*This factor (10⁴) is applicable when it is used a Hemocytometer

SEEDING DENSITY

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

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

Surface of the most common plates for culture:

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

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