

Certification date: 14 Nov 2017

ANALYSIS CERTIFICATE

Lot#: BHum16061

PRODUCT DESCRIPTION

Reference: HuMC

Product: Human Hepatocytes

Category: Cryopreserved Plateable, Cytochrome P450 inducible

Isolation date: 5 May 2016

Initial Isolation Viability (%): 88%

Storage conditions: Storage conditions: -196°C

DONOR DEMOGRAPHICS

Species	Sex	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	74	27	Ex smoker >10years	No	No

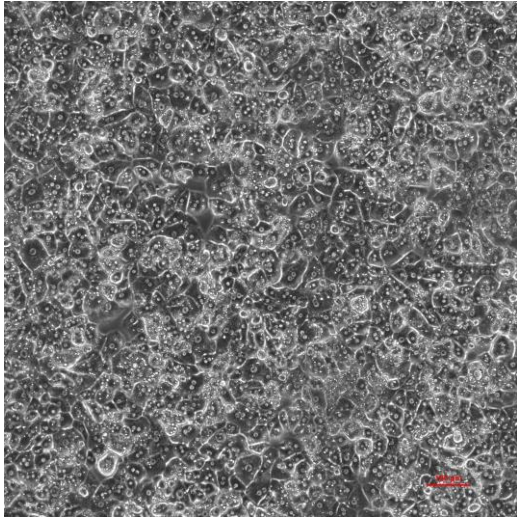
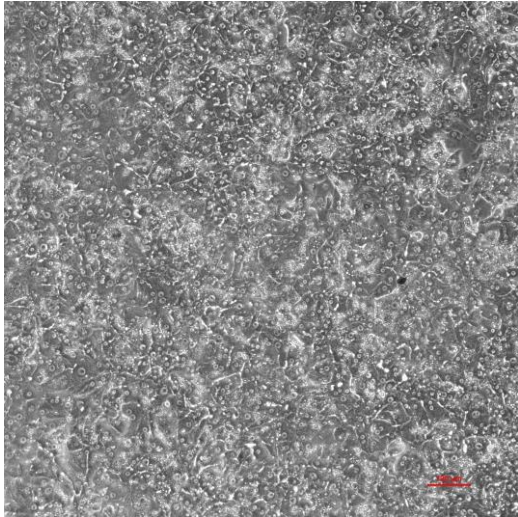
Pathology	Medications	Serological Data
Hepatic metastases	N/A	Negative tested less than 3 months before operation

Patient informed consent was obtained. The donor was serologically tested negative for following infectious diseases: HIV, Hepatitis B and C. Donor medical history were also examined prior to accepting this donor.

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CHARACTERIZATION FOR PLATEABLE CELLS

Lot#: BHum16061

Post Thaw Lot information	
Number of viable cells/vial: $7.9 \pm 1.7 \times 10^6$ (n=2) Post-thaw viability (%): 87.1 ± 1.9 (n=2)	Monolayer assessment* Plateability: YES Seeding density in 24 well recommended: 0.3×10^6 cells/well in 0.5 ml
Cell morphology 24h	Cell morphology 72h
	

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

*Resuspended human hepatocytes from the post-thaw assessment were plated in collagen-coated 24-well plates in hepatocyte plating medium. Cells were overlaid with Matrigel® (Corning) in hepatocyte maintenance medium at first medium change at day of thawing. Maintenance medium was replaced in the cultures daily.

INDUCTION FOR PLATEABLE CELLS

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

Induction (Specific Activity)				
Enzyme	Basal Activity (pmol/min/mg protein) on day 1	Basal Activity (pmol/min/mg protein) on day 3	Induced Activity (pmol/min/mg protein) on day 3	n-Fold induction
CYP1A2	12.64 ± 0.89	3.60 ± 0.30	78.37 ± 20.59	21.8
CYP2B6	9.11 ± 0.42	5.34 ± 0.32	12.80 ± 2.59	2.4
CYP3A4/5	10.22 ± 0.36	4.32 ± 0.33	11.50 ± 2.81	2.7

Cryopreserved human hepatocytes were thawed and plated on 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 [0.4% (v/v) DMSO] or inducers (Rifampicin, Omeprazole and Phenobarbital) began 1-day post-plating and continued for 48 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	430
CYP2B6	11
CYP3A4/5	247

Cryopreserved human hepatocytes were thawed, plated on 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 [0.4% (v/v) DMSO] or inducers (Rifampicin, Omeprazole and Phenobarbital) began 1-day post-plating and continued for 48 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
CYP3A4/5	Midazolam	3	30	1-Hydroxymidazolam

• **PHASE II: UGTs AND SULT ACTIVITIES 24h AFTER SEEDING EXPRESSED IN pmol/min/mg PROTEIN**

Enzyme	CONJUGATE	pmol/min/mg
UGT	7-OH coumarin glucuronide	105.95 ± 5.48
SULT	7-OH coumarin sulfate	5.99 ± 1.31

Cryopreserved human hepatocytes were thawed, plated on 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

Signed:



Quality Manager
Unit for Research
Cytes Biotechnologies

