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: -196ºC

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.

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

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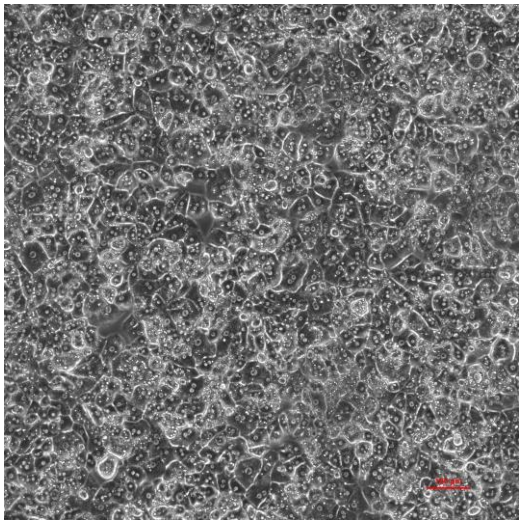
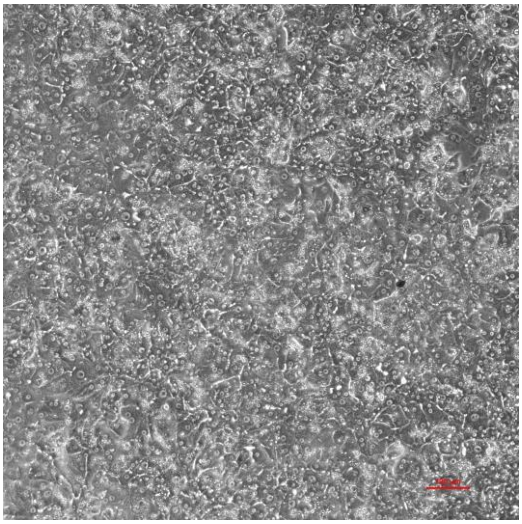
Post Thaw Lot information	
Number of viable cells/vial: $7.9 \pm 1.7*10^6$ (n=2) **Post-thaw viability (%):** 87.1 ± 1.9 (n=2)	**Monolayer assessment*** **Plateability: YES** **Seeding density in 24 well recommended:** $0.3*10^6$ cells/well in 0.5 ml
Cell morphology 24h	**Cell morphology 72h**
	

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.

- **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

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- **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

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