

## ANALYSIS CERTIFICATE

**Lot#:** BHum16059

### PRODUCT DESCRIPTION

**Reference:** HuMC

**Product:** Human Hepatocytes

**Category:** Cryopreserved Plateable, Cytochrome P450 inducible

**Isolation date:** 3 May 2016

**Initial Isolation Viability (%):** 87%

**Storage conditions:** Storage conditions: -196°C

### DONOR DEMOGRAPHICS

Species	Sex	Race	Age	BMI	Smoker	Alcohol Use	Drug Use
Human	Male	Caucasian	74	27	No	No	No

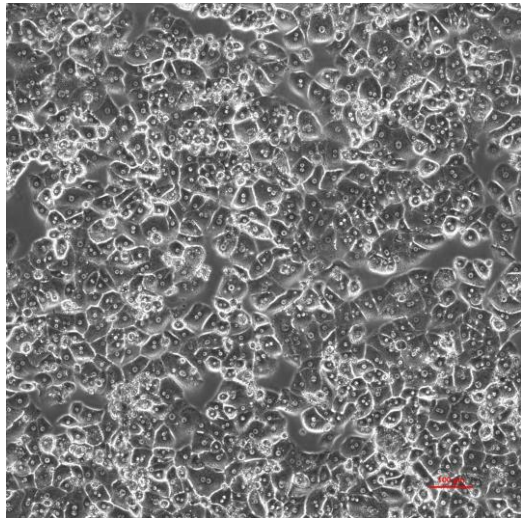
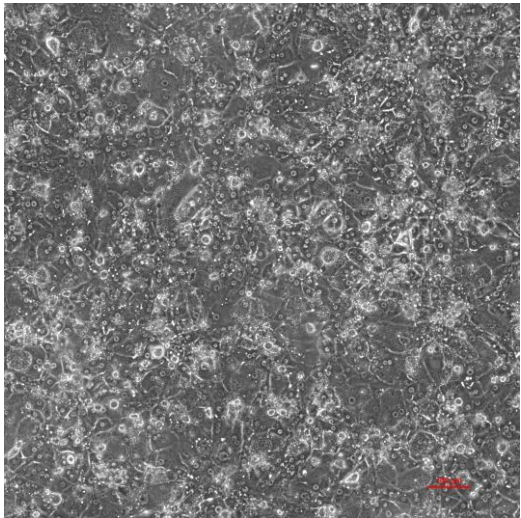
Pathology	Medications	Serological Data
Hepatic metastases	N/A	Negative tested less than 3 months before operation

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

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Post Thaw Lot information	
<b>Number of viable cells/vial:</b> $5.4 \pm 0.2 \cdot 10^6$ (n=2) <b>Post-thaw viability (%):</b> $86.5 \pm 4.5$ (n=2)	<b>Monolayer assessment*</b> <b>Plateability: YES</b> <b>Seeding density in 24 well recommended:</b> $0.3 \cdot 10^6$ 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	5.58 ± 0.22	2.53 ± 0.71	5.96 ± 0.67	2.4
CYP2B6	12.14 ± 0.09	3.35 ± 0.20	3.95 ± 0.73	1.2
CYP3A4/5	2.71 ± 0.12	0.26 ± 0.06	0.63 ± 0.13	2.5

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	35
CYP2B6	4
CYP3A4/5	166

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	316.01 ± 22.01
SULT	7-OH coumarin sulfate	15.01 ± 0.68

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

