Product Characterization Sheet
HH1085/HH1086

Human Hepatocytes, Catalog Number 82006

Classification

Grade	**Highest Quality: 999Elite™**
Plateability	**Plateable**
Viability	**>90 %**
Confluency	**>90 %**
Culture Duration	**>9 Days**
P450 Inducibility	Inducible (CYP1A2, CYP2B6, CYP3A4)
Transporter activity	CDFDA efflux qualified Pravastatin uptake qualified

Donor Demographics

Gender	Female
Age	77 years
Race	Hispanic
Cause of death	CVA 2nd to ICH
BMI	30.3
Smoking	No
Alcohol	No
Substance abuse	No
Medical history	Asthma, HTN
Infectious diseases	HBV-, HCV-, HIV-, CMV+

Post-thaw Viability and Yield

Viability	94 %
Yield	4.2 million

Storage condition: Hepatocytes are recommended to be stored at < -160°C in the vapor phase of a liquid nitrogen storage unit.

Characterization: Hepatocytes were thawed using 37°C UCRM™ and centrifuged for 10 minutes at 100g. After removing the supernatant, hepatocytes were re-suspended in UPCM™ and counted for viability and yield using the Trypan Blue exclusion method. Cells were plated in a hand-coated collagen 24-well plate at a 0.7 million cells per mL density, 0.5 mL per well, and allowed to attach 4-6 hours prior to a Matrigel® overlay.

P450 Induction

Drug Metabolizing Enzyme	Inducer (µM)	Substrate (µM)	Incubation Time (minutes)	Fold Induction (Gene Expression)	Fold Induction (Activity)
CYP1A2	Omeprazole (50)	Phenacetin (100)	30	46.41 ± 4.48	5.62 ± 0.07
CYP2B6	Phenobarbital (1000)	Bupropion (500)	30	7.72 ± 0.72	2.4 ± 0.8
CYP2C8	Rifampin (20)	Paclitaxel (20)	30	4.333 ± 1.57	
CYP2C9	Rifampin (20)	Diclofenac (25)	30	2.660 ± 0.422	
CYP2C19	Rifampin (20)	*S*-mephenytoin (250)	30	1.634 ± 0.240	
CYP3A4	Rifampin (20)	Testosterone (200)	30	24.70 ± 5.18	13.0 ± 0.3

CYP450 Induction Assessment: 96 well cultures at a cell density of 0.7 million hepatocytes/mL (56,000 hepatocytes/well) were used in the CYP450 induction assessment. The hepatocytes were cultured as collagen-Matrigel® sandwich for 1 day followed by treatment duration of 48-72 hours for mRNA and 72 hours for activity using known enzyme inducers. Induction in CYP450 activity was assessed by quantifying respective metabolite formation by LC-MS/MS. Gene expression was quantified by RT-PCR. Values reflect mean and standard deviation of triplicate treatments (N=3).

Drug Metabolism Activity

Drug Metabolizing Enzyme	Substrate (µM)	Incubation Time (minutes)	Metabolite Quantified	Activity (pmol/minute/million cells)
CYP1A2	Phenacetin (100)	15	Acetaminophen	54.7
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin	37.0
CYP2B6	Bupropion (500)	15	Hydroxybupropion	10.8
CYP2C8	Paclitaxel (20)	15	6α-Hydroxypaclitaxel	2.4
CYP2C9	Diclofenac (25)	15	4-Hydroxydiclofenac	92.2
CYP2C19	S-Mephenytoin (250)	30	4-Hydroxymephenytoin	10.0
CYP2D6	Dextromethorphan (15)	15	Dextrorphan	25.7
CYP2E1	Chlorzoxazone (250)	15	6-Hydroxychlorzoxazone	34.7
CYP3A4	Midazolam (20)	10	1-Hydroxymidazolam	44.5
	Testosterone (200)	15	6β-Hydroxytestosterone	470.7
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	102.6

UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	395.0
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	21.0

CYP450 Activity Assessment: The hepatocytes were incubated at a cell density of 0.5 million cells/mL in a 48-well plate (125,000 hepatocytes/well) for the designated time durations with isoform-selective substrates. The metabolites were identified and analyzed using LC-MS/MS.

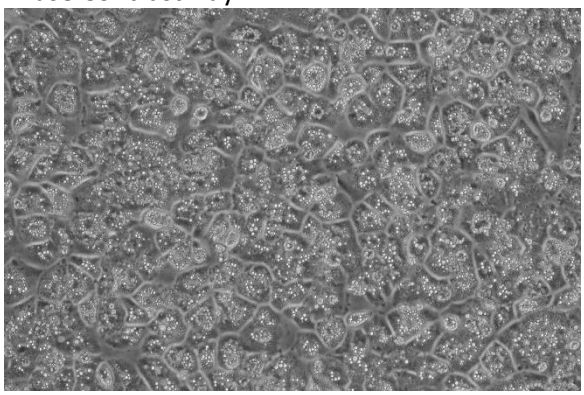
Pravastatin Uptake Transporter Activity

Pravastatin (pmol/10^6 Cells)	Pravastatin with Rifampin (pmol/10^6 Cells)	% Inhibition by Rifampin
0.812	0.31	62 %

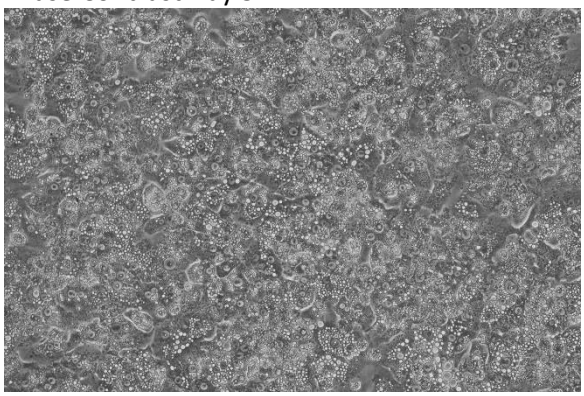
Pravastatin Uptake Assessment: 96 well cultures at a cell density of 0.7 million hepatocytes/mL (56,000 hepatocytes/well) were used in the Pravastatin Uptake Assessment. After approximately 6 hours in culture, the hepatocytes were treated with and without 20 uM Rifampin for a pre-incubation time of 30 minutes. Following pre-incubation, 25 uM pravastatin with and without rifampin was incubated for a duration time of 6 minutes. Values reflect the mean of triplicate treatments (N=3). The metabolites were identified and analyzed using LC-MS/MS.

Photomicrographs (100X, Phase Contrast)

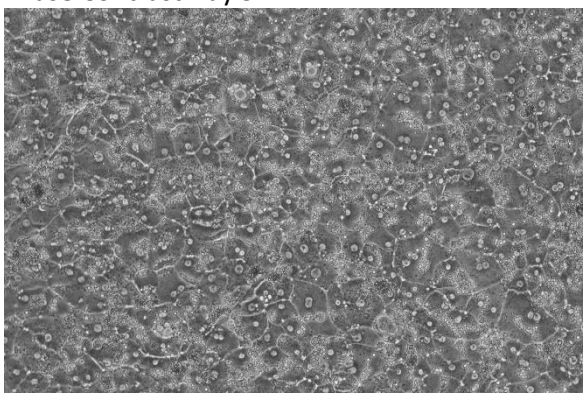
Phase Contrast Day 2



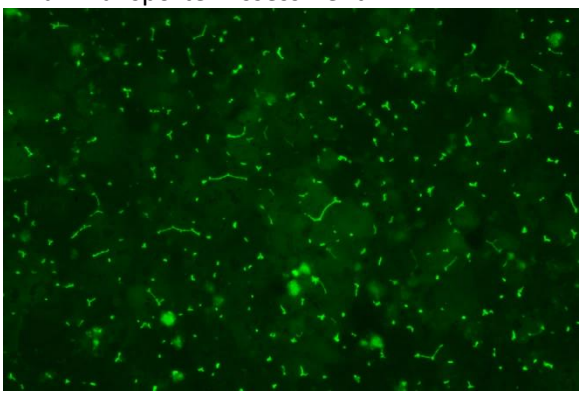
Phase Contrast Day 5



Phase Contrast Day 9



Efflux Transporter Assessment



Monolayer Comments: HH1085/HH1086 has high attachment efficiency and a confluency of 90-100 % by 24 hours. This lot exhibits excellent morphology and remains intact for over 9 days in culture.

Efflux Transporter Assessment: The hepatocytes were cultured at a cell density of 0.7 million hepatocytes/mL in a 12-well plate as a collagen-Matrigel® sandwich. On day 5, the hepatocytes were treated with incubation medium containing 5 µM carboxy-2',7' dichlorofluorescein diacetate (CDFDA) and imaged on fluorescein isothiocyanate (FITC) fluorescent filter to assess bile canalicular formation.

IVAL cell culture media and tissue culture plates used in this evaluation:

- Recovery of thawed hepatocytes - Cat. No. 81015 - UCRM™ Universal Cryopreservation Recovery Media, 50 mL tube
- Initial plating of hepatocytes - Cat. No. 81016 - UPCM™ Universal Primary Cell Plating Media, 50 mL tube
- Sandwich culture with 0.25 mg Matrigel® - Cat. No. 81018/81019 - HIM™ Hepatocyte Induction Media, 50 mL tube/500 mL bottle
- Suspension and incubation of hepatocytes - Cat. No. 81039/81040 - HQM™ Hepatocyte Incubation Media, 50 mL tube/500 mL bottle
- Collagen coated plates - Cat. No. 71006, 71008 - CellAffix™ 24-well and 96-well Collagen Hand Coated tissue culture plate, 5 plates per pack.

To inquire about our products and services or for technical questions please contact:

- In Vitro ADMET Laboratories by phone at +1 (866) 458-1094 or +1 (410) 869-9037 or email at info@invitroadmet.com