Product Characterization Sheet
HH1052

Human Hepatocytes, Catalog Number 82006

In Vitro ADMET Laboratories, Inc.

Classification

Plateability	Plateable
Number of days plateable	5 days
Confluency	85 %
P450 Inducibility	Yes
Transporter activity	Pravastatin uptake qualified
Number of donors	1

Post-thaw Viability and Yield

Viability	89 %
Yield	7.2 million

Donor Demographics

Gender	Male
Age	44 years
Race	Caucasian
Cause of death	Anoxia 2nd to CVA
BMI	24.6
Smoking	Yes
Alcohol	No
Substance abuse	No
Medical history	COPD, HTN, renal disease
Infectious diseases	HBV-, HCV-, HIV-, CMV-, EBV (IgG)+

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	Substrate (µM)	Incubation Time (minutes)	Fold Induction (Gene Expression)	Fold Induction (Activity)
CYP1A2	Omeprazole (50)	30	32.77 ± 0.88	4.66 ± 0.56
CYP2B6	Phenobarbital (1000)	30	9.26 ± 0.64	3.2 ± 0.3
CYP3A4	Rifampin (20)	30	34.79 ± 5.54	17.6 ± 3.0

CYP450 Induction Assessment: 96 well cultures at a cell density of 0.5 million hepatocytes/mL (50,000 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 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	96.4
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin	1.2
CYP2B6	Bupropion (500)	15	Hydroxybupropion	21.5
CYP2C8	Paclitaxel (20)	15	6α-Hydroxypaclitaxel	2.5
CYP2C9	Diclofenac (25)	15	4-Hydroxydiclofenac	77.0
CYP2C19	S-Mephenytoin (250)	30	4-Hydroxymephenytoin	9.8
CYP2D6	Dextromethorphan (15)	15	Dextrorphan	6.0
CYP2E1	Chlorzoxazone (250)	15	6-Hydroxychlorzoxazone	71.5
CYP3A4	Midazolam (20)	10	1-Hydroxymidazolam	1.9
	Testosterone (200)	15	6β-Hydroxytestosterone	14.9
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	70.3
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	2746.7
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	31.1

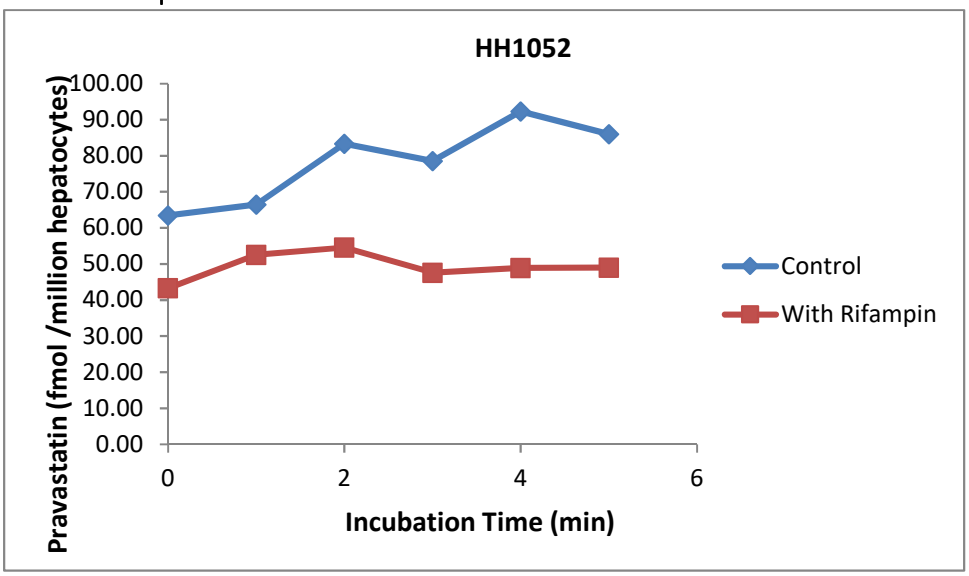
CYP450 Activity Assessment: The hepatocytes were incubated at a cell density of 0.5 million cells/mL in a 48-well plate (125,000 hepatocytes/well) for the designated time durations with isoform-selective substrates. The metabolites were identified and analyzed using LC-MS/MS.

Product Characterization Sheet
HH1052

Human Hepatocytes, Catalog Number 82006

In Vitro ADMET Laboratories, Inc.

Pravastatin Uptake Assessment

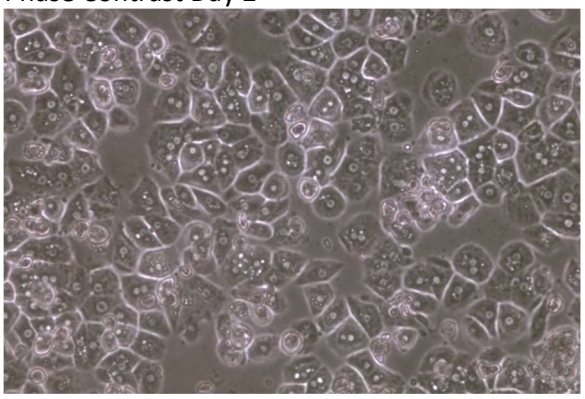


Lot HH1052	Pravastatin (fmol /million hepatocytes)					
Sample/Time (minute)	0	1	2	3	4	5
Control	63.47	66.53	83.33	78.53	92.30	86.03
With Rifampin	43.30	52.53	54.57	47.60	48.97	49.00

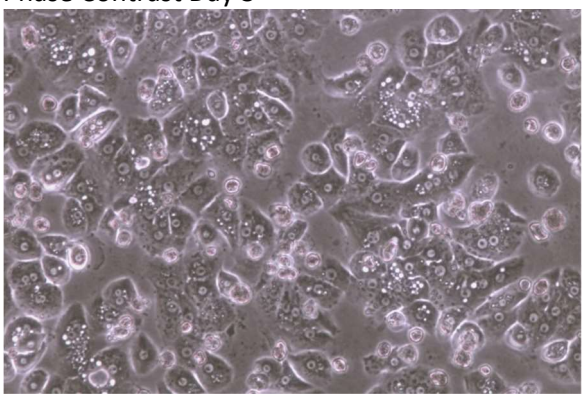
Pravastatin Uptake Assessment: 96 well cultures at a cell density of 0.5 million hepatocytes/mL (50,000 hepatocytes/well) were used in the Pravastatin Uptake Assessment. After approximately 20-24 hours in culture, the hepatocytes were treated with 1 µM of Pravastatin with and without Rifampin for a time duration of 0, 1, 2, 3, 4, and 5 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 3

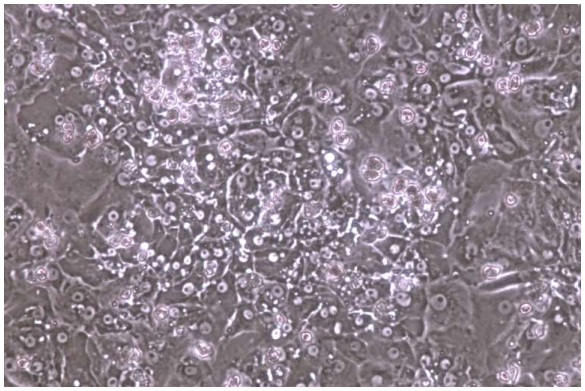


Product Characterization Sheet
HH1052

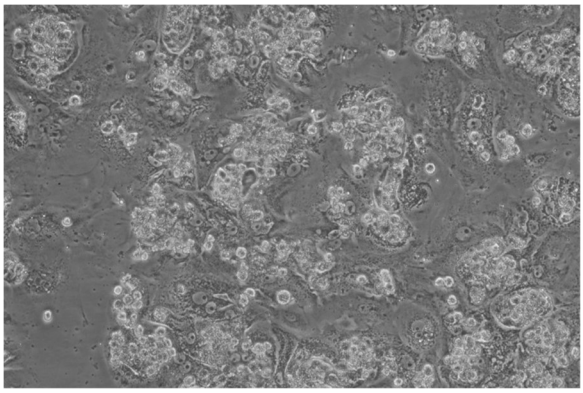
Human Hepatocytes, Catalog Number 82006

In Vitro ADMET Laboratories, Inc.

Phase Contrast Day 6



Long-term Human Plasma Incubation Day 7



Monolayer Comments: HH1052 has a good attachment efficiency and a confluency of 80% by 24 hours. This lot exhibits good morphology and remains intact for 5 days in culture.

Human Plasma Incubation: The hepatocytes were cultured at a cell density of 0.35 million hepatocytes/ 0.5 mL in a 24-well plate as a collagen-Matrigel® sandwich. On day 2, the hepatocytes were treated with media containing human plasma and received subsequent media changes every other day to assess long term effects of plasma on morphology.

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