Product Characterization Sheet
HH1119

Human Hepatocytes, Catalog Number 82006

In Vitro ADMET Laboratories, Inc.

Classification

Plateability	Plateable
Number of days plateable	5 days
Confluency	70 %
P450 Inducibility	Yes
Transporter activity	Pravastatin uptake qualified
Number of donors	1

Donor Demographics

Gender	Female
Age	64
Race	Caucasian
Cause of death	CVA 2nd to ICH
BMI	21.0
Smoking	No
Alcohol	Yes
Substance abuse	No
Medical history	Diabetes, HTN
Infectious diseases	HBV-, HCV-, HIV-, CMV+

Post-thaw Viability and Yield

Viability	87 %
Yield	5.5 million

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	18.11 ± 1.97	6.07 ± 2.23
CYP2B6	Phenobarbital (1000)	Buproprion (500)	30	6.13 ± 0.29	1.01 ± 0.05
CYP2C8	Rifampin (20)	Paclitaxel (20)	30		1.14 ± 0.16
CYP2C9	Rifampin (20)	Diclofenac (25)	30	2.63 ± 0.29	4.58 ± 0.47
CYP2C19	Rifampin (20)	*S*-mephenytoin (250)	30	0.74 ± 0.08	1.12 ± 1.22
CYP3A4	Rifampin (20)	Midazolam (20)	30		7.95 ± 2.06
	Rifampin (20)	Testosterone (200)	30	11.96 ± 1.91	1.75 ± 0.87

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 48-72 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	48.9 ± 10.2
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin	136 ± 235.4
CYP2B6	Bupropion (500)	15	Hydroxybupropion	9.34 ± 0.99
CYP2C8	Paclitaxel (20)	15	6α-Hydroxypaclitaxel	18.8 ± 3.9
CYP2C9	Diclofenac (25)	15	4-Hydroxydiclofenac	86.8 ± 3.9
CYP2C19	S-Mephenytoin (250)	30	4-Hydroxymephenytoin	1 ± 0.6
CYP2D6	Dextromethorphan (15)	15	Dextrorphan	0.58 ± 0.31
CYP2E1	Chlorzoxazone (250)	15	6-Hydroxychlorzoxazone	140 ± 19.1
CYP3A4	Midazolam (20)	10	1-Hydroxymidazolam	21 ± 3.2
	Testosterone (200)	15	6β-Hydroxytestosterone	152 ± 35
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	106 ± 18.4
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	1058 ± 123.9
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	34.5 ± 6.1

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.

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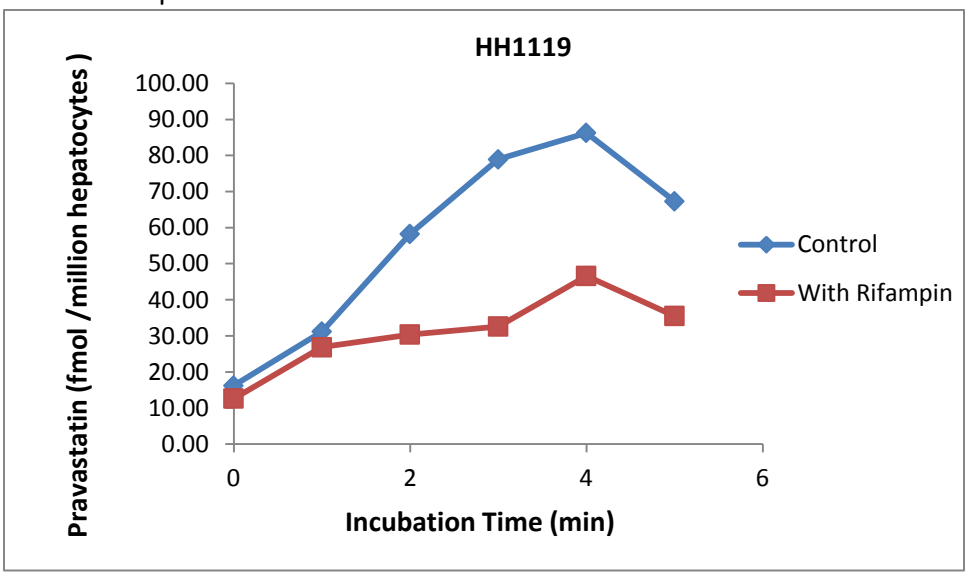
Pravastatin Uptake Assessment

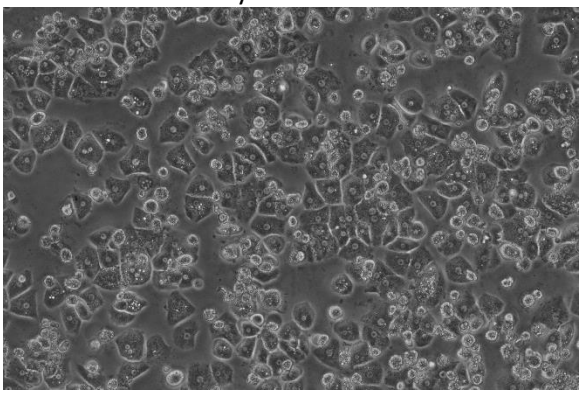


Lot HH1119	Pravastatin (fmol /million hepatocytes)					
Sample/Time (minute)	0	1	2	3	4	5
Control	16.20	31.17	58.27	78.93	86.27	67.33
With Rifampin	12.60	26.87	30.37	32.60	46.60	35.50

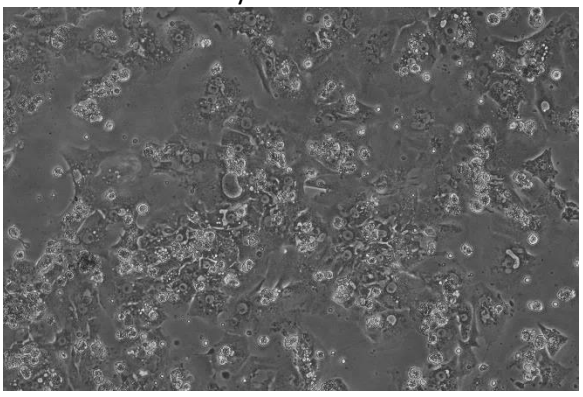
Pravastatin Uptake Assessment: 96 well cultures at a cell density of 0.5 million hepatocytes/mL (50,000 hepatocytes/well) were used in the Pravastatin Uptake Assessment. After approximately 20-24 hours in culture, the hepatocytes were treated with 1 µM of Pravastatin with and without Rifampin for a time 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 7



Monolayer Comments: HH1119 has a good attachment efficiency of 70 % and maintains a confluency of 70 % by 24 hours. Minor degradation of the monolayer is observed at day 4. This lot exhibits good morphology and remains intact for 5 days in culture.

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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