

Product Characterization Sheet

HH1125/HH1135

Human Hepatocytes, Catalog Number 82006



Classification

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

Donor Demographics

Gender	Male
Age	41
Race	Caucasian
Cause of death	Head trauma
BMI	21.7
Smoking	NA
Alcohol	NA
Substance abuse	NA
Medical history	Hypertension
Infectious diseases	HBV-, HCV-, HIV-, CMV+

Post-thaw Viability and Yield

Viability	93 %
Yield	4.4 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 4°C HQM™ and counted for viability and yield using the Trypan Blue exclusion method.

P450 Induction

Drug Metabolizing Enzyme	Substrate (μM)	Incubation Time (minutes)	Fold Induction (Gene Expression)	Fold Induction (Activity)
CYP1A2	Omeprazole (50)	30	45.95 ± 11.37	3.6 ± 0.04
CYP2B6	Phenobarbital (1000)	30	3.66 ± 0.13	1.16 ± 0.04
CYP3A4	Rifampin (20)	30	7.28 ± 1.46	1.43 ± 0.04

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)	Metabolite Quantified	Activity (pmol/minute/ million cells)
CYP1A2	Phenacetin (100)	Acetaminophen	78.2 ± 1.74
CYP2A6	Coumarin (50)	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	44.1 ± 5.29
CYP2A6	Coumarin (50)	7-Hydroxycoumarin	13.5 ± 0.91
CYP2A6	Coumarin (50)	7-Hydroxycoumarin Glucuronide	7.6 ± 1.12
CYP2A6	Coumarin (50)	7-Hydroxycoumarin Sulfate	22.9 ± 3.27
CYP2B6	Bupropion (500)	Hydroxybupropion	5.24 ± 0.42
CYP2C8	Paclitaxel (20)	6α-hydroxypaclitaxel	1.58 ± 0.07
CYP2C9	Diclofenac (25)	4-OH Diclofenac	222 ± 31.7
CYP2C19	S-Mephenytoin (250)	4-OH S-Mephenytoin	23.2 ± 4.31
CYP2D6	Dextromethorphan (15)	Dextrophan	39.9 ± 6.04
CYP2E1	Chlorzoxazone (250)	6-OH Chlorzoxazone	167 ± 7.06
CYP3A4-1	Midazolam (20)	1-Hydroxymidazolam	14.6 ± 0.36
CYP3A4-2	Testosterone (200)	6β-hydroxytestosterone	172 ± 5.05
ECOD	7-Ethoxycoumarin (100)	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	36 ± 2.89
ECOD	7-Ethoxycoumarin (100)	7-Hydroxycoumarin	13.8 ± 0.2
ECOD	7-Ethoxycoumarin (100)	7-Hydroxycoumarin Glucuronide	21 ± 1.54
ECOD	7-Ethoxycoumarin (100)	7-Hydroxycoumarin Sulfate	13.8 ± 0.2
UGT	7-Hydroxycoumarin (100)	7-Hydroxycoumarin Glucuronide	706 ± 65
SULT	7-Hydroxycoumarin (100)	7-Hydroxycoumarin Sulfate	37.1 ± 2.56

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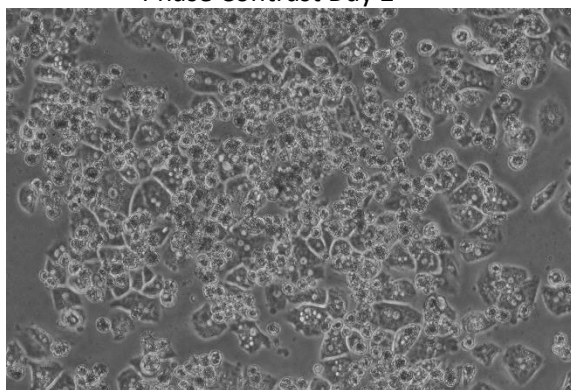


GST	Acetaminophen (10)	Acetaminophen Glutathione	37.2 ± 1.02
UGT	Acetaminophen (10)	Acetaminophen Glucuronide	175 ± 12.23
SULT	Acetaminophen (10)	Acetaminophen Sulfate	98.7 ± 5.46
FMO	Benzydamine HCl (250)	Benzydamine-N-Oxide	172 ± 48.5
MAO	Kynuramine HCl (160)	4-hydroxyquinoline	260 ± 23.7
AO	Carbazeran HCl (10)	4-Hydroxycarbazeran	0.1 ± 0.04
NAT1	4-Aminobenzoic HCl (200)	N-Acetyl-p-aminobenzoic acid	27.9 ± 1.84

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.

Photomicrographs (100X, Phase Contrast)

Phase Contrast Day 2



Monolayer Comments: HH1125 and HH1135 have a fair attachment efficiency of 60 % and maintains a confluency of 70 % by 24 hours. This lot exhibits good morphology and remains intact for 5 days in culture.

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