

Product Characterization Sheet

HH1118

Human Hepatocytes, Catalog Number 82006



Classification

Plateability	Plateable
Number of days plateable	Over 5 days
Confluency	85 %
P450 Inducibility	Yes
Transporter activity	CDFDA efflux qualified
Number of donors	1

Donor Demographics

Gender	Male
Age	44 years
Race	Hispanic
Cause of death	CVA 2 nd to ICH
BMI	23.5
Smoking	Yes
Alcohol	Yes
Substance abuse	No
Medical history	NA
Infectious diseases	HBV-, HCV-, HIV-, CMV+

Post-thaw Viability and Yield

Viability	79 %
Yield	3.6 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	33.96 ± 8.72	10.68 ± 6.30
CYP2B6	Phenobarbital (1000)	Bupropion (500)	30	59.15 ± 4.34	3.72 ± 0.32
CYP2C8	Rifampin (20)	Paclitaxel (20)	30		1.70 ± 0.68
CYP2C9	Rifampin (20)	Diclofenac (25)	30	3.10 ± 0.70	3.99 ± 0.19
CYP2C19	Rifampin (20)	S-mephenytoin (250)	30	0.74 ± 0.12	1.71 ± 0.46
CYP3A4	Rifampin (20)	Midazolam (20)	30		6.06 ± 1.27
	Rifampin (20)	Testosterone (200)	30	7.35 ± 1.77	6.70 ± 1.13

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	74.7 ± 17
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin	462 ± 39.1
CYP2B6	Bupropion (500)	15	Hydroxybupropion	98.4 ± 20
CYP2C8	Paclitaxel (20)	15	6α-Hydroxypaclitaxel	44.6 ± 12
CYP2C9	Diclofenac (25)	15	4-Hydroxydiclofenac	264 ± 29.6
CYP2C19	S-Mephenytoin (250)	30	4-Hydroxymephenytoin	20.1 ± 9.9
CYP2D6	Dextromethorphan (15)	15	Dextrorphan	62.3 ± 3.9
CYP2E1	Chlorzoxazone (250)	15	6-Hydroxychlorzoxazone	7.1 ± 5.3
CYP3A4	Midazolam (20)	10	1-Hydroxymidazolam	137 ± 10.4
	Testosterone (200)	15	6β-Hydroxytestosterone	743 ± 77
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	196 ± 20.9
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	1631 ± 394
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	34.7 ± 8.6

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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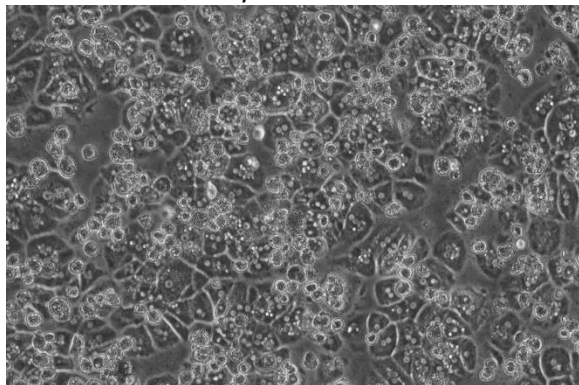
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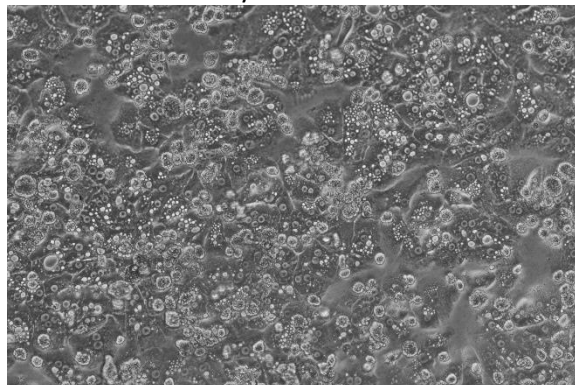


Photomicrographs (100X, Phase Contrast)

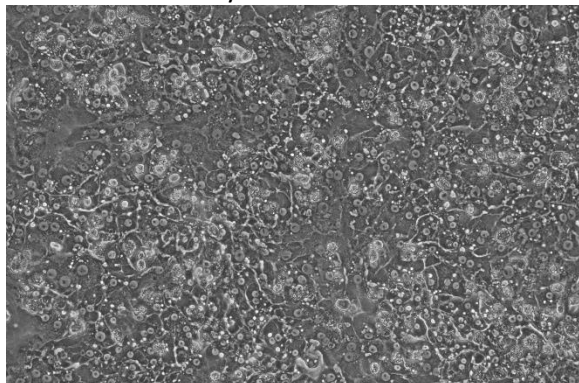
Phase Contrast Day 2



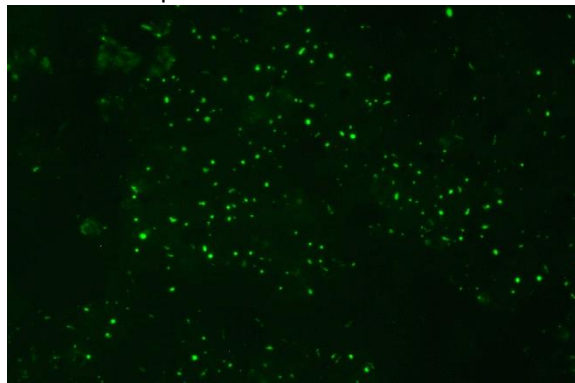
Phase Contrast Day 3



Phase Contrast Day 7



Efflux Transporter Assessment



Monolayer Comments: HH1118 has a good attachment efficiency of 70 % and a confluency of 85 % by 24 hours. This lot exhibits excellent morphology and remains intact for over 5 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 μ M 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