Product Characterization Sheet
HH1110

Human Hepatocytes, Catalog Number 82006

In Vitro ADMET Laboratories, Inc.

Classification

Plateability	Plateable
Number of days plateable	1 day
Confluency	70 %
P450 Inducibility	No
Transporter activity	No
Number of donors	2

Donor Demographics

Gender	Male
Age	47
Race	Hispanic
Cause of death	CVA 2nd to ICH
BMI	25.2
Smoking	Yes
Alcohol	Yes
Substance abuse	No
Medical history	Diabetes, HTN,
Infectious diseases	HBV-, HCV-, HIV-, CMV+

Post-thaw Viability and Yield

Viability	83 %
Yield	6.1 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.

Drug Metabolism Activity

Drug Metabolizing Enzyme	Substrate (μM)	Incubation Time (minutes)	Metabolite Quantified	Activity (pmol/minute/million cells)
CYP1A2	Phenacetin (100)	15	Acetaminophen	20 ± 2.5
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin	234.5 ± 20.4
CYP2B6	Bupropion (500)	15	Hydroxybupropion	35.1 ± 3.4
CYP2C8	Paclitaxel (20)	15	6α-Hydroxypaclitaxel	51.6 ± 5
CYP2C9	Diclofenac (25)	15	4-Hydroxydiclofenac	137 ± 8
CYP2C19	S-Mephenytoin (250)	30	4-Hydroxymephenytoin	3 ± 0.9
CYP2D6	Dextromethorphan (15)	15	Dextrorphan	10.8 ± 1.2
CYP2E1	Chlorzoxazone (250)	15	6-Hydroxychlorzoxazone	30 ± 5.4
CYP3A4	Midazolam (20)	10	1-Hydroxymidazolam	14 ± 0.6
	Testosterone (200)	15	6β-Hydroxytestosterone	116 ± 40.8
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	121.7 ± 1.1
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	664 ± 43
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	34.6 ± 5.3

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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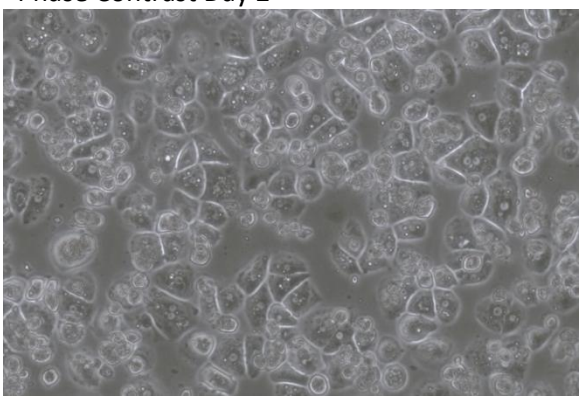
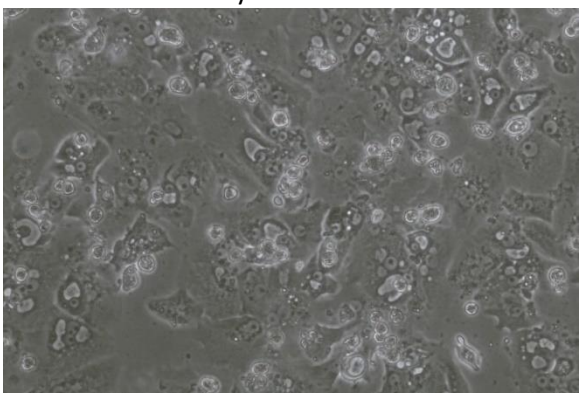
Photomicrographs (100X, Phase Contrast)

Phase Contrast Day 2



Phase Contrast Day 5



Monolayer Comments: HH1110 has a good attachment efficiency and a confluency of 70 % by 24 hours. HH1110 attachment is improved by plating hepatocytes at a density of 1.0 million cells per mL. This lot exhibits deterioration of the monolayer by day 3 and is recommended for short term 2 day studies.

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