

Product Characterization Sheet

HH1128

Human Hepatocytes, Catalog Number 82006



Classification

Plateability	Plateable
Number of days plateable	5 days
Confluency	90 %
P450 Inducibility	Yes
Transporter activity	No
Number of donors	1

Donor Demographics

Gender	Male
Age	38 years
Race	Caucasian
Cause of death	CVA 2 nd to ICH
BMI	22.7
Smoking	No
Alcohol	No
Substance abuse	No
Medical history	Bells palsy
Infectious diseases	HBV-, HCV-, HIV-, CMV+

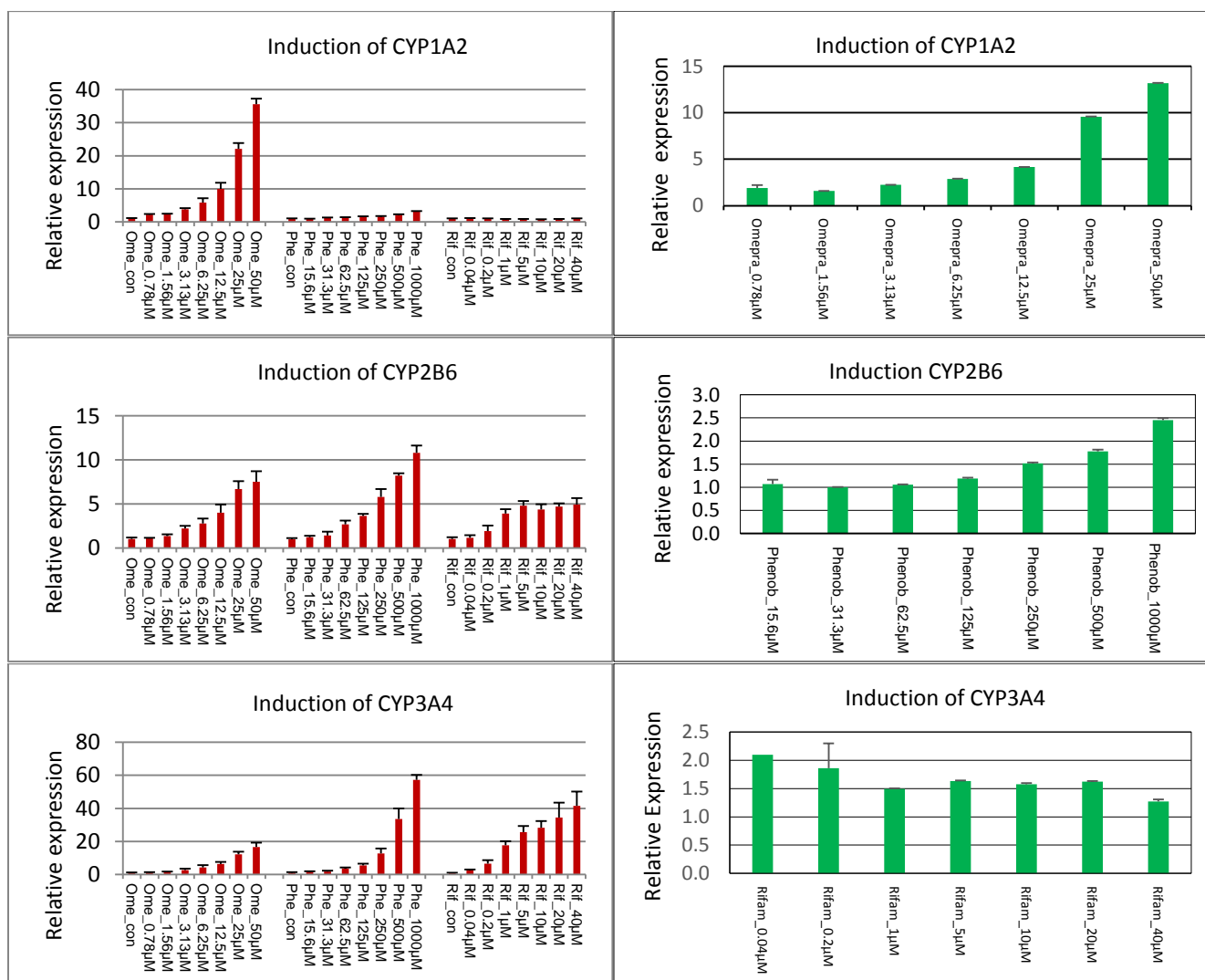
Post-thaw Viability and Yield

Viability	90 %
Yield	5.9 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 Fold Induction (Gene Expression)

P450 Fold Induction (Enzyme Activity)



Product Characterization Sheet

HH1128

Human Hepatocytes, Catalog Number 82006



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	11.3 ± 8.1
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin Glucuronide	11 ± 4
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin Sulfate	27 ± 9.97
CYP2B6	Bupropion (500)	15	Hydroxybupropion	-0.13 ± 0.31
CYP2C8	Paclitaxel (20)	15	6α-hydroxypaclitaxel	0.66 ± 0.19
CYP2C9	Diclofenac (25)	15	4-OH Diclofenac	130 ± 3.97
CYP2C19	S-Mephenytoin (250)	30	4-OH S-Mephenytoin	2.88 ± 0.19
CYP2D6	Dextromethorphan (15)	15	Dextrophan	17.7 ± 4.16
CYP2E1	Chlorzoxazone (250)	15	6-OH Chlorzoxazone	233 ± 105
CYP3A4-1	Midazolam (20)	10	1-Hydroxymidazolam	1.08 ± 0.18
CYP3A4-2	Testosterone (200)	15	6β-hydroxytestosterone	38.4 ± 3.08
ECOD	7-Ethoxycoumarin (100)	30	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	81.4 ± 7.39
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	10.8 ± 0.97
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin Glucuronide	59.6 ± 5.61
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin Sulfate	10.96 ± 0.82
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin Glucuronide	648 ± 55.8
SULT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin Sulfate	38.4 ± 4.11
FMO	Benzydamine HCl (250)	30	Benzydamine-N-Oxide	60.5 ± 10.1
MAO	Kynuramine HCl (160)	30	4-hydroxyquinoline	319 ± 61.3
AO	Carbazeran HCl (10)	30	4-Hydroxycarbazeran	16.3 ± 1.44
NAT1	4-Aminobenzoic HCl (200)	30	N-Acetyl-p-aminobenzoic acid	11.3 ± 0.84
NAT2	Sulfamethazine (100)	30	N-Acetyl-sulfamethazine	8.62 ± 0.47

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

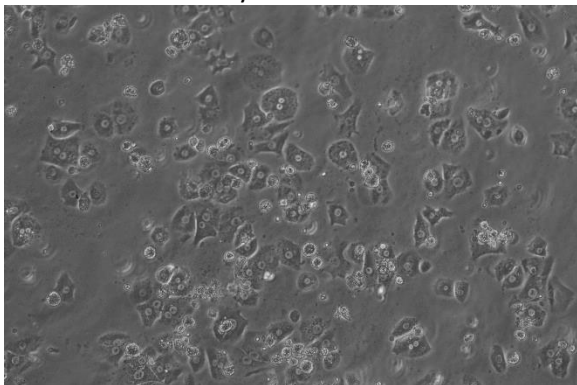
HH1128

Human Hepatocytes, Catalog Number 82006

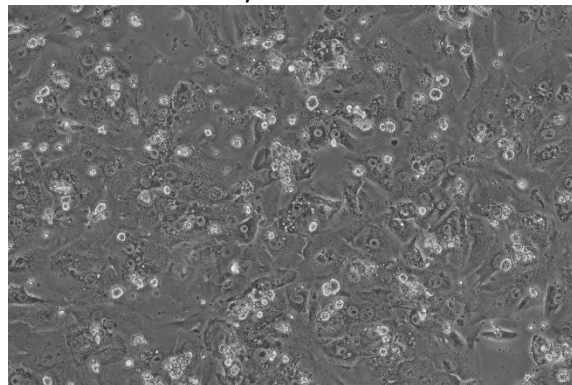


Photomicrographs (100X, Phase Contrast)

Phase Contrast Day 2



Phase Contrast Day 6



Monolayer Comments: It is recommended to seed HH1128 at a seeding density of 0.7 million cells per mL but with an additional 10 – 20% higher volume. HH1128 has a fair attachment efficiency of 60 % and a confluency of 90 % by day 6. This lot remains intact for over 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