

# Product Characterization Sheet

## HH1101/HH1112/HH1120

Human Hepatocytes, Catalog Number 82006



### Classification

Plateability	Plateable
Number of days plateable	3 days
Confluency	60 %
P450 Inducibility	Yes
Transporter activity	No
Number of donors	1

### Donor Demographics

Gender	Male
Age	19
Race	African American
Cause of death	Head trauma 2 <sup>nd</sup> to GSW
BMI	22.8
Smoking	No
Alcohol	Yes
Substance abuse	No
Medical history	Hemophilia, chest trauma
Infectious diseases	HBV-, HCV-, HIV-, CMV+

### Post-thaw Viability and Yield

Viability	85 %
Yield	4.1 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.

### P450 Induction

Drug Metabolizing Enzyme	Inducer (µM)	Substrate (µM)	Incubation Time (minutes)	Fold Induction (Gene Expression)	Fold Induction (Activity)
CYP1A2	Omeprazole (25)	Phenacetin (100)	30	55.56 ± 5.27	6.36 ± 0.44
CYP2B6	Phenobarbital (1000)	Bupropion (500)	30	48.36 ± 2.79	2.33 ± 0.98
CYP3A4	Rifampin (20)	Testosterone (200)	30	50.67 ± 10.37	282 ± 34.6

**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	245 ± 22.87
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin Glucuronide	96.6 ± 41.6
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin Sulfate	318 ± 57
CYP2B6	Bupropion (500)	15	Hydroxybupropion	2.84 ± 0.13
CYP2C8	Paclitaxel (20)	15	6α-hydroxypaclitaxel	6.51 ± 5.68
CYP2C9	Diclofenac (25)	15	4-OH Diclofenac	222 ± 24.67
CYP2C19	S-Mephenytoin (250)	30	4-OH S-Mephenytoin	69.28 ± 8.63
CYP2D6	Dextromethorphan (15)	15	Dextrophan	8.23 ± 0.31
CYP2E1	Chlorzoxazone (250)	15	6-OH Chlorzoxazone	94.8 ± 13.7
CYP3A4-1	Midazolam (20)	10	1-Hydroxymidazolam	30.6 ± 2.46
CYP3A4-2	Testosterone (200)	15	6β-hydroxytestosterone	358 ± 36.1
ECOD	7-Ethoxycoumarin (100)	30	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	105.8 ± 6.49
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	20 ± 1.55
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin Glucuronide	37.3 ± 1.68
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin Sulfate	48.56 ± 3.29
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin Glucuronide	1068 ± 119
SULT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin Sulfate	89.2 ± 23.2

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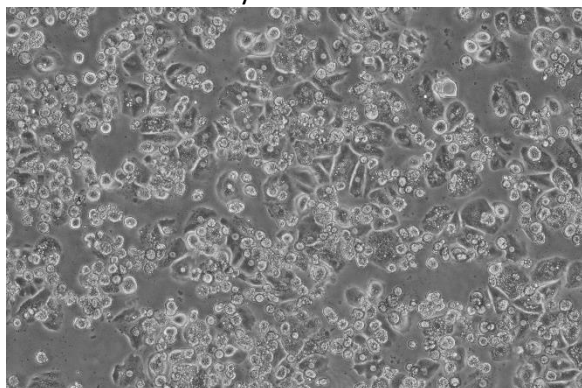


FMO	Benzydamine HCl (250)	30	Benzydamine-N-Oxide	74.1 ± 3.2
MAO	Kynuramine HCl (160)	30	4-hydroxyquinoline	555 ± 105
AO	Carbazeran HCl (10)	30	4-Hydroxycarbazeran	23.7 ± 4.68
NAT1	4-Aminobenzoic HCl (200)	30	N-Acetyl-p-aminobenzoic acid	18.6 ± 0.6
NAT2	Sulfamethazine (100)	30	N-Acetyl-sulfamethazine	45.4 ± 3.67

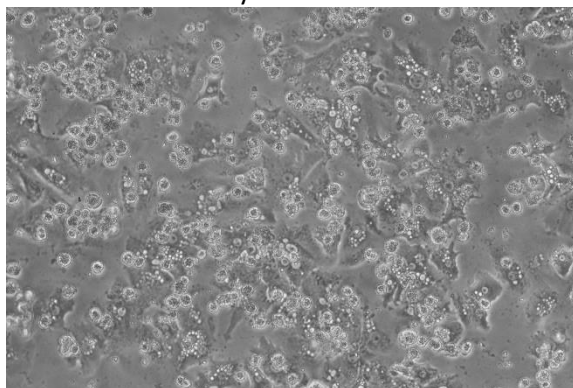
**CYP450 Activity Assessment:** The hepatocytes were incubated at a cell density of 0.5 million cells/mL in a 48-well plate (125,000 hepatocytes/well) for the designated time durations with isoform-selective substrates. The metabolites were identified and analyzed using LC-MS/MS.

### Photomicrographs (100X, Phase Contrast)

Phase Contrast Day 2



Phase Contrast Day 4



**Monolayer Comments:** HH1101, 1112, 1120 has a good attachment efficiency of 70 % and maintains a confluency of 60 % by 24 hours. Minor degradation of the monolayer is observed at day 4. This lot exhibits good morphology and remains intact for 3 days in culture. Although the lot is recommended for 3 days in culture, it remains highly inducible.

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](mailto:info@invitroadmet.com)