

Product Characterization Sheet

PHH8015A

Human Hepatocytes, Catalog Number 82005



Classification

Plateability	Plateable
Number of days plateable	7 Days
Confluency	85%
P450 Inducibility	TBD
Transporter activity	NA
Number of donors	10

Post-thaw Viability and Yield

Viability	86.0 %
Yield	6.72 million

Donor Demographics

	HH1085	HH1121	HH1134	HH1128	HH1087
Gender	Female	Female	Female	Male	Female
Age	77 years	24 years	46	38 years	44
Race	Hispanic	Hispanic	Hispanic	Caucasian	Caucasian
Cause of death	CVA 2 nd to ICH	CVA 2 nd to ICH	CVA 2 nd to ICH	CVA 2 nd to ICH	Anoxia 2 nd to drug intoxication
BMI	30.3	34.0	25.2	22.7	35.6
Smoking	No	No	No	No	No
Alcohol	No	Yes	No	No	No
Substance abuse	No	Yes	No	No	No
Medical history	Asthma, HTN	HTN	Thyroid disorder	Bells palsy	NA
Infectious diseases	HBV-, HCV-, HIV-, CMV+, EBV (IgG)+	HBV-, HCV-, HIV-, CMV+, EBV (IgG)+	HBV-, HCV-, HIV-, CMV+, EBV IgG+	HBV-, HCV-, HIV-, CMV+, EBV (IgG)+	HBV-, HCV-, HIV-, CMV-

	HH1045	HH1072	HH1125	HH1113	HH1076
Gender	Male	Female	Male	Male	Male
Age	9 years	40 years	41	44 years	34 years
Race	Hispanic	Caucasian	Caucasian	Hispanic	Caucasian
Cause of death	Brain tumor	CVA	Head trauma	CVA 2 nd to ICH	Head trauma 2 nd to blunt injury
BMI	19.5	37.3	21.7	23.5	35.6
Smoking	No	Yes	NA	Yes	Yes
Alcohol	No	Yes	NA	Yes	Yes
Substance abuse	No	No	NA	No	Yes
Medical history	Asthma	Asthma, epilepsy	Hypertension	NA	Asthma
Infectious diseases	HBV-, HCV-, HIV-, CMV-, EBV (IgG)+	HBV-, HCV-, HIV-, CMV-, EBV (IgG)+	HBV-, HCV-, HIV-, CMV+, EBV IgG+, EBNA+	HBV-, HCV-, HIV-, CMV+, EBV (IgG)+	HBV-, HCV-, HIV-, CMV+

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.

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P450 Induction

Drug Metabolizing Enzyme	Substrate (μM)	Incubation Time (minutes)	Fold Induction (Gene Expression)	Fold Induction (Activity)
CYP1A2	Omeprazole (50)	30	TBD	TBD
CYP2B6	Phenobarbital (1000)	30	TBD	TBD
CYP3A4	Rifampin (20)	30	TBD	TBD

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	TBD
CYP2A6	Coumarin (50)	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	TBD
CYP2A6	Coumarin (50)	7-Hydroxycoumarin	TBD
CYP2A6	Coumarin (50)	7-Hydroxycoumarin Glucuronide	TBD
CYP2A6	Coumarin (50)	7-Hydroxycoumarin Sulfate	TBD
CYP2B6	Bupropion (500)	Hydroxybupropion	TBD
CYP2C8	Paclitaxel (20)	6α-hydroxypaclitaxel	TBD
CYP2C9	Diclofenac (25)	4-OH Diclofenac	TBD
CYP2C19	S-Mephenytoin (250)	4-OH S-Mephenytoin	TBD
CYP2D6	Dextromethorphan (15)	Dextrophan	TBD
CYP2E1	Chlorzoxazone (250)	6-OH Chlorzoxazone	TBD
CYP3A4-1	Midazolam (20)	1-Hydroxymidazolam	TBD
CYP3A4-2	Testosterone (200)	6β-hydroxytestosterone	TBD
ECOD	7-Ethoxycoumarin (100)	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	TBD
ECOD	7-Ethoxycoumarin (100)	7-Hydroxycoumarin	TBD
ECOD	7-Ethoxycoumarin (100)	7-Hydroxycoumarin Glucuronide	TBD
ECOD	7-Ethoxycoumarin (100)	7-Hydroxycoumarin Sulfate	TBD
UGT	7-Hydroxycoumarin (100)	7-Hydroxycoumarin Glucuronide	TBD
SULT	7-Hydroxycoumarin (100)	7-Hydroxycoumarin Sulfate	TBD
GST	Acetaminophen (10)	Acetaminophen Glutathione	TBD
UGT	Acetaminophen (10)	Acetaminophen Glucuronide	TBD
SULT	Acetaminophen (10)	Acetaminophen Sulfate	TBD
FMO	Benzydamine HCl (250)	Benzydamine-N-Oxide	TBD
MAO	Kynuramine HCl (160)	4-hydroxyquinoline	TBD
AO	Carbazepan HCl (10)	4-Hydroxycarbazepan	TBD
NAT1	4-Aminobenzoic HCl (200)	N-Acetyl-p-aminobenzoic acid	TBD

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.

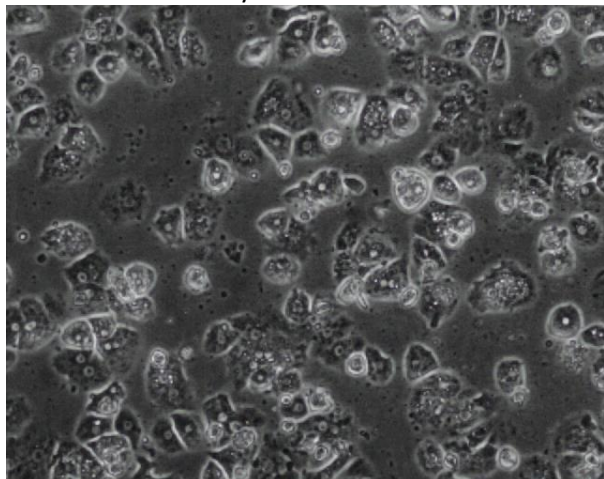
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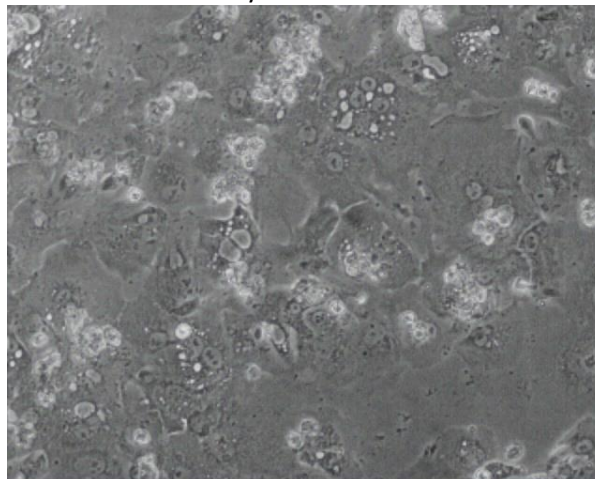
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Photomicrographs (100X, Phase Contrast)
Phase Contrast Day 2



Phase Contrast Day 7



Monolayer Comments: PHH8015A has a fair attachment efficiency of 85 % and maintains a confluency of xx % by 24 hours. This lot exhibits good morphology and 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
- Suspension and incubation of hepatocytes - Cat. No. 81039/81040 - HQM™ Hepatocyte Incubation Media, 50 mL tube/500 mL bottle

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