

Product Characterization Sheet

PHP8002

Human Hepatocytes, Catalog Number 82005



Classification

Suspension	X
Metabolism	X
Inducible	X
CDFDA Efflux	-
Long Term Culture	-
Confluency	70-80 %
Number of Donors	5

Donor Demographics

Gender	Male and Female
Age	9 - 55
Race	Mixed
Cause of Death	N/A
BMI	19.5 – 44.9
Smoking	Mixed
Alcohol	Mixed
Substance	Mixed
Medical Background	N/A
Serological Data	CMV-
HIV	Negative

Post-thaw

Viability	94 %
Yield	6.9 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 Induction

Enzyme	Fold Induction mRNA	Fold Induction Activity
CYP1A2	TBD	27.7
CYP2B6	TBD	7.6
CYP3A4	TBD	10.2

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 quantitating respective metabolite formation by LC-MS/MS using an API 3000 triple-quadrupole mass spectrometer coupled to Agilent 1100 HPLC. Gene expression was quantified by RT-PCR. Values reflect mean and standard deviation of triplicate treatments (N=3).

Drug Metabolism Activity

Enzyme	Metabolite Measured	Activity pmol/million cells/min
CYP1A2	Acetaminophen	35.3 ± 1.1
CYP2A6	Coumarin	128.9 ± 5.04
CYP2B6	Hydroxybupropion	18.9 ± 0.27
CYP2C8	6α-hydroxy paclitaxel	1.62 ± 0.23
CYP2C9	4-hydroxydiclofenac	194.5 ± 11.9
CYP2C19	4'-hydroxymephenytoin	6.0 ± 0.36
CYP2D6	Dextrorphan	10.2 ± 0.41
CYP2E1	6-hydroxychlorzoxazone	55.4 ± 1.7
CYP3A4	1-hydroxymidazolam	36.4 ± 5.3
	6β-hydroxy testosterone	344.3 ± 6.45
ECOD	7-Hydroxycoumarin	92.5 ± 4.97
UGT	7-Hydroxycoumarin glucuronide	1094.5 ± 40.6
Sulfotransferase	7-Hydroxycoumarin sulfate	45.95 ± 2.7

CYP450 Activity Assessment: The hepatocytes were incubated at a cell density of 0.5 million enterocytes/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 API 3000 mass spectrometer connected to Agilent 1100 series HPLC.

Product Characterization Sheet

PHP8002

Human Hepatocytes, Catalog Number 82005

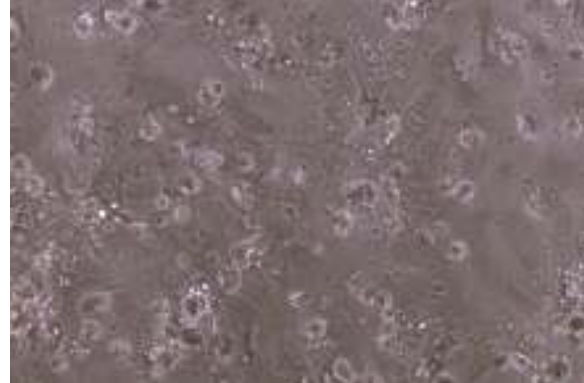


Photomicrographs (100X, Phase Contrast)

Phase Contrast Day 3



Phase Contrast Day 3



Monolayer Comments: PHH8002 has a good attachment efficiency and a confluency of 70% by 24 hours. This lot exhibits good morphology and remains intact for over a week 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