

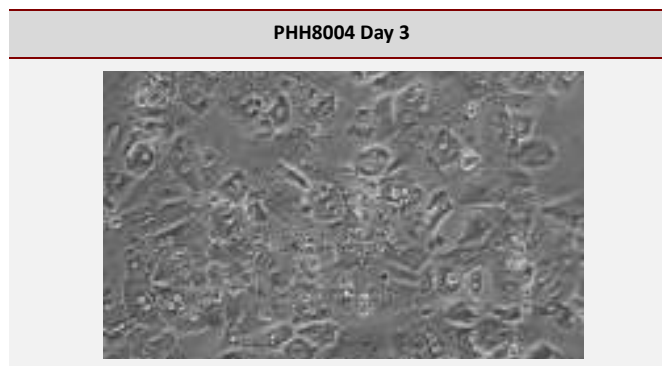
Product Characterization Sheet

PCS PHH8004

Human Hepatocytes



Classification		
<ul style="list-style-type: none"> • Pooled • 5-Donor • Plateable 		
Catalog Number	Lot Number	Species
82005	PHH8004	Human



Donors	Sex	Age	BMI	Ethnicity	Cause of Death	Smoking	Alcohol	Substance	Serological Data	HIV
HH1007	Female	26	26.9	Caucasian	GSW-Head	Yes	Yes	Yes	CMV+	Negative
HH1023	Female	21	23.2	Caucasian	Anoxia-Drug Intoxication	Yes	Yes	Yes	CMV-	Negative
HH1033	Female	40	44.9	Caucasian	CVA	Yes	Yes	Yes	CMV-	Negative
HH1036	Male	55	26.0	Caucasian	Head Trauma	Yes	Yes	No	CMV-	Negative
HH1057	Female	33	21.1	Caucasian	Stroke	No	No	No	CMV-	Negative

PHH8004 Post-thaw Viability and Yield				
Thawing medium	Optimal centrifuge conditions	Plating medium	% Viability	Viable cell yield per vial
UCRM™	100 x g for 10 minutes	UPCM™	88 %	5.8 x 10 ⁶ cells

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 UPCM™ for plating or 4°C HQM™ for suspension incubations, and counted for viability and yield using the Trypan Blue exclusion method.

P450 Induction Assessment Fold-Induction						
mRNA	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Average
Inducer	HH1007	HH1023	HH1033	HH1036	HH1057	Mathematical Average
CYP1A2 (50 µM Omeprazole)	32.6	127.3	35.6	76.2	35.0	61.3
CYP2B6 (1000 µM Phenobarbital)	11.5	13.3	6.7	6.3	10.9	9.7
CYP3A4 (20 µM Rifampicin)	15.8	6.9	8.9	21.2	16.1	13.8

96 well cultures at a cell density of 0.5 million hepatocytes/mL (50,000 hepatocytes/well) were used in the assessment. The hepatocytes were cultured as collagen-Matrigel® sandwich for 1 day followed by treatment duration of 48 hours for mRNA. Gene expression was quantified by RT-PCR. Values reflect mean and standard deviation of triplicate treatments (N=3).

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PHH8004 Metabolic Activity Assessment					
Metabolic Pathway	Substrate	Concentration	Incubation (min.)	Marker Metabolite	Metabolic Activity (pmol/ 10 ⁶ cells/min.)
CYP1A2	Phenacetin	100 µM	15	Acetaminophen	41.6 ± 5.1
CYP2B6	Bupropion	500 µM	15	Hydroxybupropion	34.9 ± 1.3
CYP2C8	Paclitaxel	20 µM	15	6α-hydroxy paclitaxel	34.6 ± 0.6
CYP2C9	Diclofenac	25 µM	15	4-hydroxydiclofenac	192.8 ± 7.1
CYP2C19	S-Mephenytoin	250 µM	30	4'-hydroxymephenytoin	17.8 ± 1.5
CYP2D6	Dextromethorphan	15 µM	15	Dextrorphan	19.3 ± 1.0
CYP2E1	Chlorzoxazone	250 µM	15	6-hydroxychlorzoxazone	71.4 ± 3.5
CYP3A4	Testosterone	200 µM	15	6β-hydroxy testosterone	544.2 ± 28.8
CYP3A4	Midazolam	20 µM	10	1'-hydroxymidazolam	25.8 ± 0.2
ECOD	7-Ethoxycoumarin	100 µM	30	7-Hydroxycoumarin, 7-Hydroxycoumarin sulfate, 7-Hydroxycoumarin glucuronide	83.8 ± 3.0
UGT	7-Hydroxycoumarin	100 µM	30	7-Hydroxycoumarin glucuronide	584.7 ± 29.2
Sulfotransferase	7-Hydroxycoumarin	100 µM	30	7-Hydroxycoumarin sulfate	38.2 ± 1.2

The hepatocytes were incubated at a cell density of 0.5 million hepatocytes/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.

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 medium - Cat. No. 81039 - HQM™ Hepatocyte Incubation Media, 50 mL tube

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