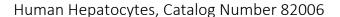
Product Characterization Sheet HH1099





Classification

Plateability	Plateable	
Number of days plateable	5 days	
Confluency	65 %	
P450 Inducibility	No	
Transporter activity	No	
Number of donors	1	

Post-thaw Viability and Yield

Viability	88 %	
Yield	5.0 million	

Donor Demographics

<u> </u>			
Gender	Male		
Age	46		
Race	Caucasian		
Cause of death	CVA 2 nd to ICH		
ВМІ	41.4		
Smoking	No		
Alcohol	Yes		
Substance abuse	No		
Medical history	Diabetes, HTN		
Infectious diseases	HBV-, HCV-, HIV-, CMV-		

Characterization: Hepatocytes were thawed using 37°C UCRMTM and centrifuged for 10 minutes at 100g. After removing the supernatant, hepatocytes were re-suspended in UPCMTM 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.

Drug Metabolism Activity

Drug Metabolizing Enzyme	Substrate (μΜ)	Incubation Time (minutes)	Metabolite Quantified	Activity (pmol/minute/million cells)
CYP1A2	Phenacetin (100)	15	Acetaminophen	30.8 ± 7.8
CYP2A6	Coumarin (50)	30	7-Hydroxycoumarin	11 ± 0.2
CYP2B6	Bupropion (500)	15	Hydroxybupropion	6.6 ± 2.1
CYP2C8	Paclitaxel (20)	15	6α-Hydroxypaclitaxel	17.6 ± 4.8
CYP2C9	Diclofenac (25)	15	4-Hydroxydiclofenac	97 ± 2.5
CYP2C19	S-Mephenytoin (250)	30	4-Hydroxymephenytoin	5.7 ± 1.1
CYP2D6	Dextromethorphan (15)	15	Dextrorphan	18.8 ± 4.7
CYP2E1	Chlorzoxazone (250)	15	6-Hydroxychlorzoxazone	57.6 ± 2.8
CYP3A4	Midazolam (20)	10	1-Hydroxymidazolam	37.7 ± 4.1
	Testosterone (200)	15	6β-Hydroxytestosterone	372 ± 136
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	22 ± 1.4
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	458 ± 65
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	22.3 ± 0.2

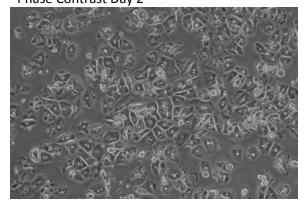
<u>CYP450 Activity Assessment:</u> The hepatocytes were incubated at a cell density of 0.5 million cells/mL in a 48-well plate (125,000 hepatocytes/well) for the designated time durations with isoform-selective substrates. The metabolites were identified and analyzed using LC-MS/MS.

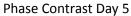
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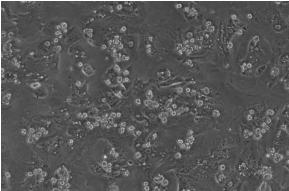
Human Hepatocytes, Catalog Number 82006



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







Monolayer Comments: HH1099 has a fair attachment efficiency and a confluency of 65 % by 24 hours. HH1099 attachment is improved by plating hepatocytes at a density of 1.0 million cells per mL. Stretching of the hepatocytes in the monolayer is observed, however this lot exhibits good morphology and remains intact for 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