Product Characterization Sheet

PCS 031-225

Rat Hepatocytes

Catalog Number	Lot Number	Strain
82018	031- 225	Sprague Dawley

Sex	Age/Weight	Infections/Diseases
Male	8 weeks/200-250 g	Negative

Classification
• **Plateable**

Post-thaw Viability and Plating Condition					
Thawing medium	**% Viability**	**Viable cell yield per vial**	**Plating medium**	**Well format**	**Optimal seeding density**
UCRM™	90 %	3.8 x 10^6 cells	UPCM™	24-well	0.7 x 10^6 cells/mL, 0.5 mL/well

Hepatocytes were thawed using 37°C UCRM™ and centrifuged for 5 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 plate and allowed to attach 4-6 hours prior to a Matrigel® overlay.

Phase I and Phase II Assessment				
Metabolic Pathway	**Substrate**	**Concentration**	**Incubation (min.)**	**Metabolic Activity (pmol/10^6 cells/min.)**
ECOD	7-Ethoxycoumarin	100 µM	30	238.19 ± 20.05
UGT	7-Hydroxycoumarin	100 µM	30	1250.25 ± 216.96
Sulfotransferase	7-Hydroxycoumarin	100 µM	30	64.91 ± 22.46

The hepatocytes were incubated at a cell density of 0.5 million hepatocytes/mL in a 12-well plates 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.

P450 Induction Assessment			
Enzyme	**Inducer**	**Concentration**	**Fold-induction mRNA**
CYP1A1	3-Methylcholantherene	1 µM	TBD
CYP2B1	Phenobarbital	500 µM	TBD
CYP3A1	Dexamethasone	2 µM	TBD

The hepatocytes were incubated at a cell density of 0.6 million hepatocytes per mL, in 96-well plates, for 4 hrs followed by an overnight Matrigel® overlay. The hepatocytes were then treated with designated inducers for 3 days following which, cells were harvested for mRNA. Gene expression for selected P450 isoforms was determined via RT-PCR.

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. 81037/81038 - RHIM™ Rodent Hepatocyte Induction Media, 50 mL tube/500 mL bottle
- Collagen coated plates - Cat. No. 71006 - CellAffix™ 24-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

In Vitro ADMET Laboratories, LLC

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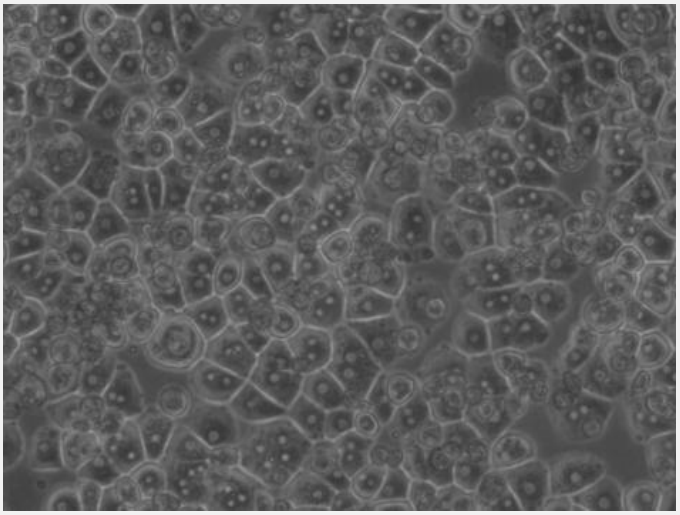
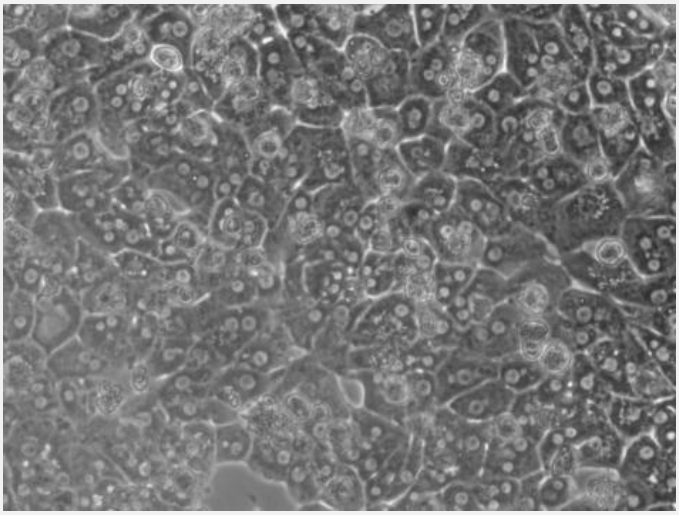
Monolayer Assessment	
Initial Attachment	80 %, Initial media change and overlay applied at 4-6 hours
Monolayer Confluency at 24 hours	90 %, Monolayer formation complete by day 2
Monolayer Comments	High attachment efficiency Some loss of monolayer integrity observed by day 4

Photomicrographs (24-well, 100X, Phase Contrast)

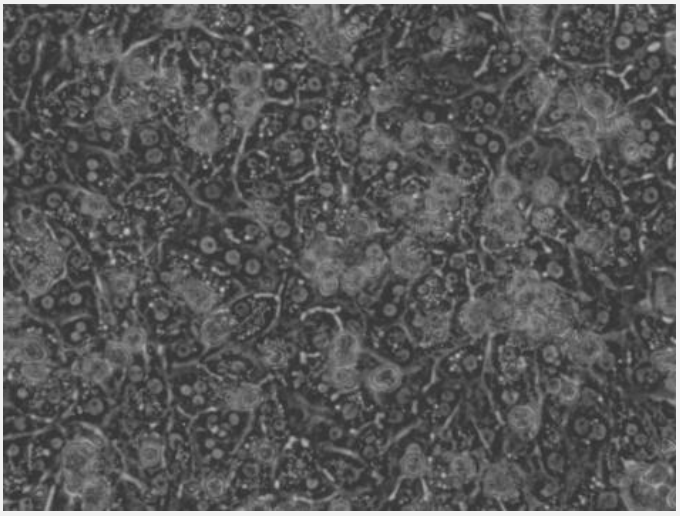
Initial attachment



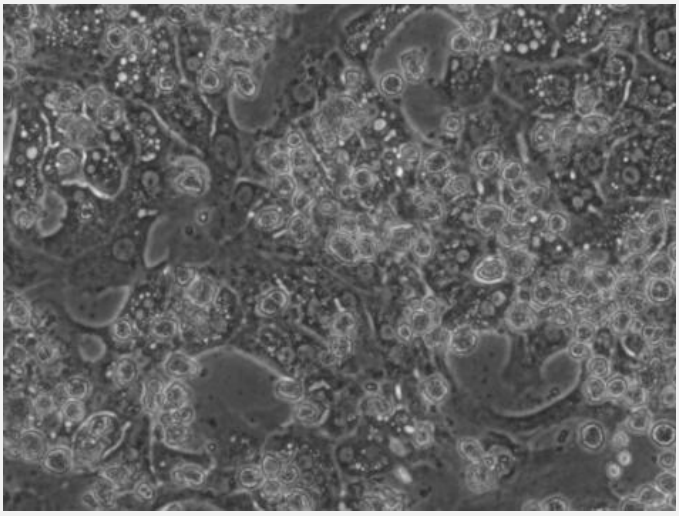
24 Hours post plating



3 Days post plating



4 Days post plating



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