

Product Characterization Sheet

PCS 151-227

Rat Hepatocytes

Catalog Number	Lot Number	Strain	Classification		
82026	151-227	Wistar Han	• Plateable		
Sex	Age/Weight	Infections/Diseases			
Male	8 weeks/200-250 g	Negative			
Post-thaw Viability and Plating Condition					
Thawing medium	% Viability	Viable cell yield per vial	Plating medium	Well format	Optimal seeding density
UCRM™	87 %	6.1 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	263.85 ± 16.65
UGT	7-Hydroxycoumarin	100 µM	30	1320.81 ± 41.49
Sulfotransferase	7-Hydroxycoumarin	100 µM	30	101.74 ± 1.64

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-Methylcholanthrene	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

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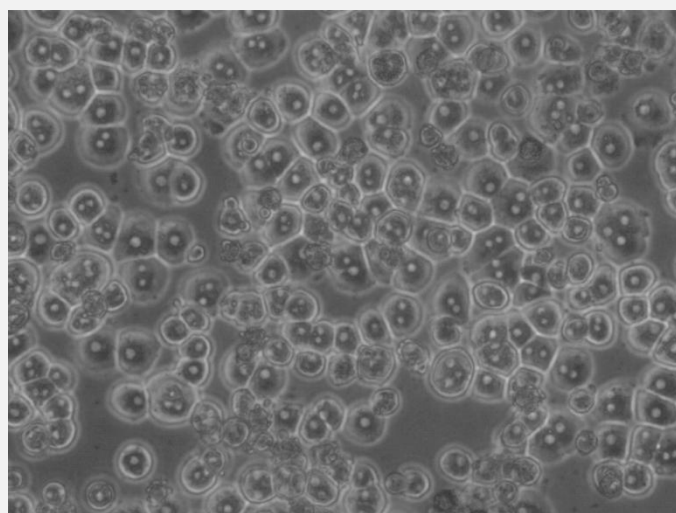
Rat Hepatocytes

Monolayer Assessment

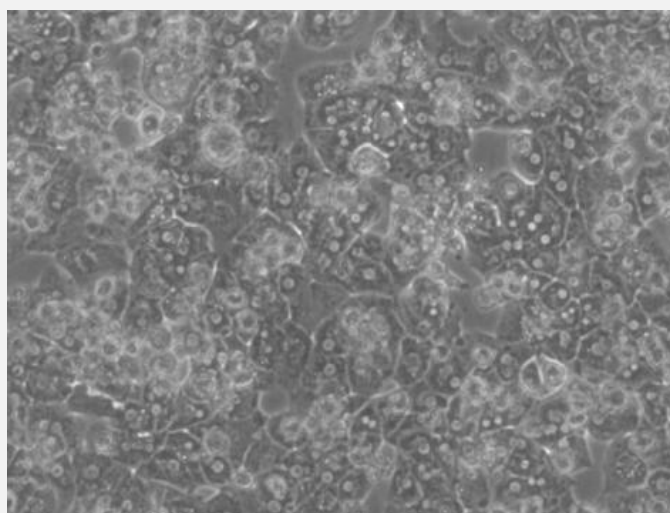
Initial Attachment	60 %, Initial medium change and overlay applied at 4 hours
Monolayer Confluency at 24 hours	80 %, Chord formation observed by day 2
Monolayer Comments	Monolayer intact although losing membrane integrity by day 4/5

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

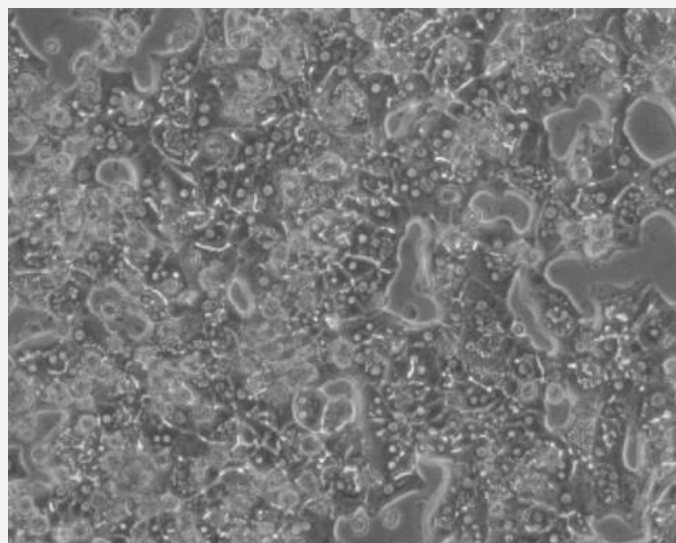
Initial attachment



24 Hours post plating



3 Days post plating



4 Days post plating

