

Product Characterization Sheet

031-234

Rat Hepatocytes, Catalog Number 82018



Classification

Plateability	Plateable
Number of days plateable	5
Confluency	80 %
P450 Inducibility	Yes
Number of donors	5

Lot Demographics

Gender	Male
Strain	Sprague Dawley
Age	7-10 weeks
Weight	200-250 g
Infectious diseases	Negative

Post-thaw Viability and Yield

Viability	93 %
Yield	4.7 million

Characterization: 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 at a density of 0.7 million cells per mL in a hand-coated collagen plate and allowed to attach 4-6 hours prior to a Matrigel® overlay.

Drug Metabolism Activity

Drug Metabolizing Enzyme	Substrate (μM)	Incubation Time (minutes)	Metabolite Quantified	Activity (pmol/minute/million cells)
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	255 ± 25.3
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	1474 ± 111
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	84 ± 4

CYP450 Activity Assessment: The hepatocytes were incubated at a cell density of 0.5 million hepatocytes/mL in a 12-well plate (500,000 hepatocytes/well) for the designated time durations with isoform-selective substrates. The metabolites were identified and analyzed using LC-MS/MS.

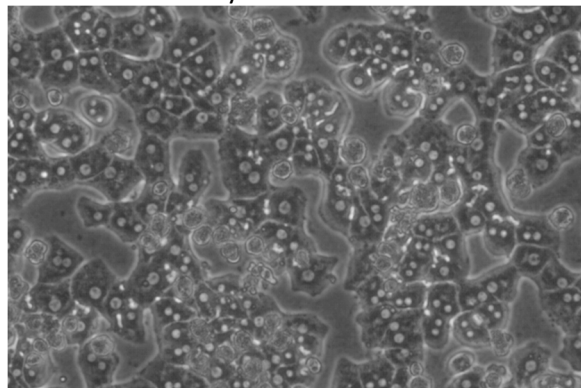
P450 Induction

Drug Metabolizing Enzyme	Inducer (μM)	Fold Induction (Gene Expression)
Cyp1a	3-Methylcholanthrene (1)	272
Cyp2b	Phenobarbital (500)	1490
Cyp3a	Dexamethasone (2)	935

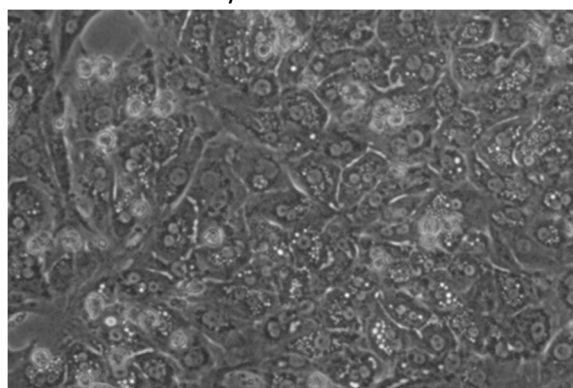
CYP450 Induction Assessment: 96 well cultures at a cell density of 0.5 million hepatocytes/mL (50,000 hepatocytes/well) were used in the CYP450 induction assessment. The hepatocytes were cultured as collagen-Matrigel® sandwich for 1 day followed by treatment duration of 48 hours for mRNA using known enzyme inducers. After the treatment period, cells were harvested and gene expression was quantified by RT-PCR. Values reflect mean and standard deviation of triplicate treatments (N=3).

Photomicrographs (100X, Phase Contrast)

Phase Contrast Day 2



Phase Contrast Day 4



Monolayer Comments: 031-234 has a fair attachment efficiency at 60 % and continues to develop a monolayer confluency of 80 % by day 3. This lot exhibits good morphology and remains intact for 5 days in culture.

Product Characterization Sheet

031-221

Rat Hepatocytes, Catalog Number 82018



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™ IVAL Universal Primary Cell Plating Medium, 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
- 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 - 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