Product Characterization Sheet 031-220





Classification

Plateability	Plateable		
Number of days plateable	5		
Confluency	90 %		
P450 Inducibility	NA		
Number of donors	7		

Lot Demographics

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

Post-thaw Viability and Yield

Viability	90 %	
Yield	4.7 million	

<u>Characterization:</u> 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.

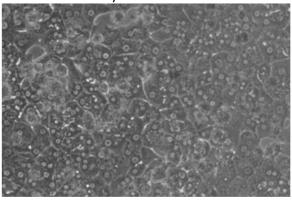
Drug Metabolism Activity

Drug Metabolizing Enzyme	Substrate (μΜ)	Incubation Time (minutes)	Metabolite Quantified	Activity (pmol/minute/million cells)
ECOD	7-Ethoxycoumarin (100)	30	7-Hydroxycoumarin	254.84 ±15.45
UGT	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin glucuronide	1415.46 ± 105.12
Sulfotransferase	7-Hydroxycoumarin (100)	30	7-Hydroxycoumarin sulfate	46.40 ± 7.13

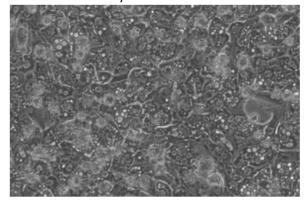
<u>CYP450 Activity Assessment:</u> The hepatocytes were incubated at a cell density of 0.5 million hepatocytes/mL in a 12-well plate (500,000 hepatocytes/well) for the designated time durations with isoform-selective substrates. The metabolites were identified and analyzed using LC-MS/MS.

Photomicrographs (100X, Phase Contrast)

Phase Contrast Day 2



Phase Contrast Day 4



Monolayer Comments: 031-220 has a good attachment efficiency at 70 % and continues to develop a monolayer confluency of 90 % by day 3. 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™ 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