Product Characterization Sheet RSE3083





Classification

Plateability	Suspension	
Number of days plateable	NA	
Confluency	NA	
Number of donors	12	

Donor Demographics

Gender	Male	
Strain	Sprague Dawley	
Age	7-10 weeks	
Infectious diseases	diseases Negative	

Post-thaw Viability and Yield

Viability	54 %	
Yield	1.6 million	

 $\underline{\text{Characterization:}} \text{ Enterocytes were thawed using 37°C CERM}^{\text{TM}} \text{ and centrifuged for 10 minutes at 100g. After removing the supernatant, enterocytes were re-suspended in 4°C HQM}^{\text{TM}} \text{ and counted for viability and yield using the Trypan Blue exclusion method.}$

Drug Metabolism Activity

Drug Metabolizing Enzyme	Substrate (μM)	Incubation Time (minutes)	Metabolite Quantified	Activity (pmol/minute/ million cells)
CYP1A2	Phenacetin (100)	15	Acetaminophen	150 ± 27.9
CYP2A6	Coumarin (50)	15	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	0.25 ± 0.11
CYP2A6	Coumarin (50)	15	7-Hydroxycoumarin	0.24 ± 0.12
CYP2A6	Coumarin (50)	15	7-Hydroxycoumarin Glucuronide	0.011 ± 0.005
CYP2A6	Coumarin (50)	15	7-Hydroxycoumarin Sulfate	0 ± 0
CYP2B6	Bupropion (500)	15	Hydroxybuproprion	0.31 ± 0.1
CYP2C8	Paclitaxel (20)	15	6α-hydroxypaclitaxel	0 ± 0
CYP2C9	Diclofenac (25)	15	4-OH Diclofenac	0.99 ± 0.03
CYP2C19	S-Mephenytoin (250)	15	4-OH S-Mephenytoin	0 ± 0
CYP2D6	Dextromethorphan (15)	15	Dextrophan	0 ± 0
CYP2E1	Chlorzoxazone (250)	15	6-OH Chlorzoxazone	4.41 ± 0.04
CYP3A4-1	Midazolam (20)	15	1-Hydroxymidazolam	0.044 ± 0.001
CYP3A4-2	Testosterone (200)	15	6β-hydroxytestosterone	8.36 ± 4.91
ECOD	7-Ethoxycoumarin (100)	15	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide	150 ± 171
ECOD	7-Ethoxycoumarin (100)	15	7-Hydroxycoumarin	150 ± 171
ECOD	7-Ethoxycoumarin (100)	15	7-Hydroxycoumarin Glucuronide	0.083 ± 0.02
ECOD	7-Ethoxycoumarin (100)	15	7-Hydroxycoumarin Sulfate	0 ± 0
UGT	7-Hydroxycoumarin (100)	15	7-Hydroxycoumarin Glucuronide	0.2 ± 0.03
SULT	7-Hydroxycoumarin (100)	15	7-Hydroxycoumarin Sulfate	0.085 ± 0.001
GST	Acetaminophen (10)	15	Acetaminophen Glutathione	0.09 ± 0.03
UGT	Acetaminophen (10)	15	Acetaminophen Glucuronide	0.15 ± 0.09
SULT	Acetaminophen (10)	15	Acetaminophen Sulfate	0.036 ± 0.012
FMO	Benzydamine HCl (250)	15	Benzydamine-N-Oxide	3.34 ± 0.3
MAO	Kynuramine HCl (160)	15	4-hydroxyquinoline	81.8 ± 11.22
AO	Carbazeran HCl (10)	15	4-Hydroxycarbazeran	0.024 ± 0.005
NAT1	4-Aminobenzoic HCl (200)	15	N-Acetyl-p-aminobenzoic acid	0 ± 0
NAT2	Sulfamethazine (100)	15	N-Acetyl-sulfamethazine	1.79 ± 0.24
1A1	7-EROD (100)	15	Resozufin	0.089 ± 0.017
2J2	Astemizole (50)	15	O-Demethyl Astemizole	7.19 ± 0.63
CES2	Irinotecan (50)	15	SN38	3.41 ± 0.07

In Vitro ADMET Laboratories | Columbia, MD 410-869-9037 | Malden, MA 781-397-9300 | info@invitroadmet.com | www.invitroadmet.com

Product Characterization Sheet RSE3083





<u>CYP450 Activity Assessment:</u> The enterocytes were incubated at a cell density of 1.5 million enterocytes/mL in a 96-well plate (150,000 enterocytes/well) for the designated time duration of 15 minutes with isoform-selective substrates. The metabolites were identified and analyzed using LC-MS/MS.

<u>Enterocyte Morphology:</u> Enterocyte cell size is approximately 12 – 17 microns in diameter. Lymphocytes are approximately 4 microns and may also be observed in the enterocyte cell population.

IVAL cell culture media and tissue culture plates used in this evaluation:

- Recovery of thawed enterocytes Cat. No. 81056 CERM™ Cryopreserved Enterocyte Recovery Media, 50 mL tube
- Suspension and incubation of enterocytes Cat. No. 81039 HQM™ Hepatocyte and Enterocyte Incubation Media, 50 mL tube

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