XENOTECH
A BioIVT Company

Data Sheet

CryostaX

Single Freeze Pooled Plateable Cryopreserved Human Hepatocytes

HPCH10+ Lot No. 2010236

Pool of 10

Assured Minimum Yield:	5.0 x 10^6 cells per vial
Viability:	84%

Values for enzyme activities were determined at a single substrate concentration and are mean ± standard deviation of three or more determinations.

Enzyme	*Marker Substrate Reaction*	*[S] (µM)*	*Rate (pmol/million cells/min)*
CYP1A2	Phenacetin *O*-dealkylation	100	9.47 ± 0.67
CYP2B6	Bupropion hydroxylation	500	13.5 ± 1.2
CYP2C8	Amodiaquine *N*-dealkylation	20	27.1 ± 0.58
CYP2C9	Tolbutamide hydroxylation	150	4.89 ± 0.30
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	400	7.14 ± 0.15
CYP2D6	Dextromethorphan *O*-demethylation	75	10.5 ± 1.1
CYP3A4/5	Midazolam 1'-hydroxylation	30	23.0 ± 1.6
UGT	7-Hydroxycoumarin glucuronidation	100	133 ± 3
SULT	7-Hydroxycoumarin sulfonation	100	8.25 ± 0.48

To measure cytochrome P450 (CYP), UDP-glucuronosyl transferase (UGT) and sulfotransferase (SULT) activities, cultured human hepatocytes (~ 20 hours post plating) were incubated in triplicate at 37 ± 2°C for 30 or 45 minutes (substrate dependent) in OptiIncubate media and marker substrate, at the final concentrations indicated. Metabolite formation was determined by validated LC-MS/MS methods with deuterated metabolites as internal standards.

Donor Information

Gender:	Males (5), Female (5)
Age:	10-67 years of age
Race:	Caucasian (9), Hispanic (1)
Cause of Death:	Anoxia (2), Cerebrovascular accident (3), Head trauma (5)
Cytomegalovirus (CMV):	Positive (6), Negative (4)
Human Immunodeficiency Virus (HIV):	Negative (10)
Hepatitis B Surface Antigen (HbsAg):	Negative (10)
Antibody to Hepatitis C Virus (HCV):	Negative (10)

Store in liquid nitrogen, vapor phase

CAUTION: This sample should be considered as a potential biohazard and universal precautions should be followed. Intended for *in vitro* use only.

This data sheet serves as a Certificate of Analysis and has been approved by Stephanie Helmstetter, Senior Manager.
Signature and Date: Stephanie Helmstetter 19 April 2022

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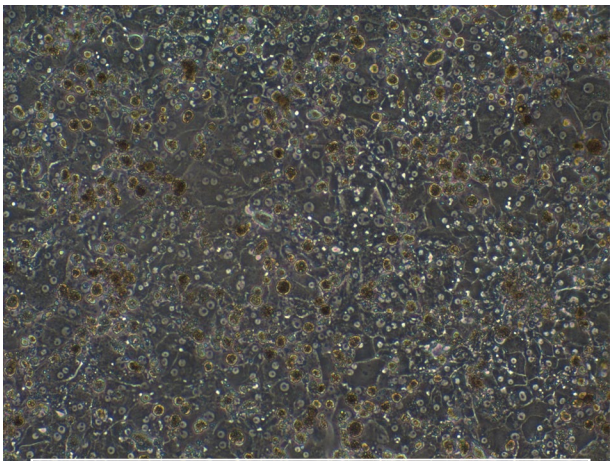
Uptake Transporter	*Marker Substrate*	*[S] (µM)*	*Uptake Ratio*
OATP1B1	Estrone sulfate	0.1	7.84
OATP1B3	CCK-8	0.1	4.60
OCT1	MPP+	0.1	7.60
NTCP	TCA	0.1	5.18

To measure uptake activities in cultured human hepatocytes (~4 hours post plating), hepatocytes were incubated in triplicate at 37 ± 2°C for 1 minute in OptiIncubate media and marker substrate, at the final concentrations indicated. Uptake was determined by liquid scintillation counter. Protein determinations were performed post incubation via isolation of microsomal fractions and protein measurements using the Pierce BCA assay.

Uptake ratio refers to the difference between uptake activities between trace substrate and excess substrate.

Induction Data

Enzyme	Inducer	mRNA Fold Induction	Marker Substrate Reaction	Enzymatic Fold Induction
CYP1A2	Omeprazole (50 µM)	29.9	Phenacetin O-dealkylation	41.3
CYP2B6	Phenobarbital (750 µM)	7.7	Bupropion hydroxylation	5.5
CYP2B6	CITCO (100 nM)	8.6	Bupropion hydroxylation	4.8
CYP3A4	Rifampin (20 µM)	5.7	Midazolam 1'-hydroxylation	4.7



Lot 2010236 on Day 4 of culture

Plate Format	Recommended Seeding Density (million cells/mL)	Recommended Seeding/ Feeding Volume Per Well
6-well format	1.3	1.7 mL
12-well format	1.3	650 µL
24-well format	1.3	330 µL
48-well format	0.75	200 µL
96-well format	Not Recommended	

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