SEKISUI XENOTECH

HEPATOSURE™

Data Sheet

HCP100.H15 Lot No. 1810179

Pool of 100 (50 Males and 50 Females)

Assured Minimum Yield:	5.0 x 10^6 cells per vial
Viability:	83%

This product was pooled from individual human hepatocytes that have been frozen and thawed. The yield and viability given above are based on experiments performed at XenoTech using our thawing protocol that includes a density gradient fractionation and the K8500 OptiThaw Kit. Details of XenoTech's hepatocyte thawing protocol can be found at www.xenotech.com. It is recommended to use XenoTech's thawing protocol, which includes a density gradient step, to maximize the viability of the recovered cells.

Enzyme	*Marker Substrate Reaction*	*[S] (µM)*	*Rate (pmol/million cells/min)*
CYP1A2	Phenacetin *O*-dealkylation	100	33.7 ± 2.6
CYP2A6	Coumarin 7-hydroxylation	50	43.1 ± 3.8
CYP2B6	Bupropion hydroxylation	500	27.3 ± 1.0
CYP2C8	Amodiaquine *N*-dealkylation	20	234 ± 23
CYP2C9	Diclofenac 4'-hydroxylation	100	147 ± 6
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	400	10.6 ± 0.4
CYP2D6	Dextromethorphan *O*-demethylation	80	32.9 ± 1.5
CYP2E1	Chlorzoxazone 6-hydroxylation	500	64.9 ± 3.2
CYP3A4/5	Testosterone 6β-hydroxylation	250	192 ± 19
CYP3A4/5	Midazolam 1'-hydroxylation	30	48.3 ± 5.0
UGT	7-Hydroxycoumarin glucuronidation	100	306 ± 14
SULT	7-Hydroxycoumarin sulfonation	100	18.5 ± 0.4

Values for enzyme activities were determined at a single substrate concentration run with triplicate determinations.

To measure cytochrome P450 (CYP), UDP-glucuronosyl transferase (UGT) and sulfotransferase (SULT) activities, hepatocytes (1 x 10^6 cells/mL) in suspension were incubated in triplicate at 37 ± 1°C for 30 minutes in OptiIncubate 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 (50), Females (50)
Age:	8-74 years of age
Race:	Caucasian (81), African American (7), Asian (4), Hispanic (8)
Cause of Death:	Anoxia (29), Head trauma (25), Cerebrovascular accident (46)
Anitbody to Cytomegalovirus (CMV):	Positive (64), Negative (36)
Human Immunodeficiency Virus (HIV):	Negative (100)
Hepatitis B Surface Antigen (HbsAg):	Negative (100)
Antibody to Hepatitis C Virus (HCV):	Negative (100)

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.

Data sheet prepared 23 August 2018

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Representative Aldehyde Oxidase Activity Data for Hepatosure

Enzyme	*Marker Substrate Reaction*	*[S] (µM)*	*Rate (pmol/million cells/min)*
Aldehyde Oxidase	*p*-Vanillin oxidation	20	444 ± 40
Aldehyde Oxidase	Phthalazine oxidation	20	148 ± 5

To measure Aldehyde Oxidase activities, hepatocytes (0.5 x 10^6 cells/mL) in suspension were incubated in triplicate at 37 ± 1°C for 30 minutes in Krebs-Henseleit buffer and marker substrate, at the final concentrations indicated. Metabolite formation was determined by validated LC-MS/MS methods.

Clearance Data

Enzyme	*Marker Substrate*	*[S] (µM)*	*CL_{int} (µL/million cells/min)*
CYP1A2	Phenacetin	1	4.3
CYP2C8	Repaglinide	0.5	6.3
CYP2C9	Tolbutamide	1.0	1.9
CYP2D6	Dextromethorphan	0.5	6.5
CYP3A4/5	Midazolam	0.5	9.9

To measure Intrinsic Clearance, hepatocytes (0.5 x 10^6 cells/mL) in suspension were incubated in triplicate at 37 ± 1°C 0-120 minutes (time points of 0, 30, 60, 90 and 120 min) in Krebs-Henseleit buffer and marker substrate, at the final concentrations indicated. Parent loss was determined by validated LC-MS/MS methods.

Uptake Activity Data

Uptake Transporter	*Marker Substrate*	*[S] (µM)*	*Rate (pmol/million cells/min)*
OATP1B1	Estrone sulfate	1	14.2
OATP1B3	CCK-8	1	2.0
OCT1	MPP+	1	6.1
NTCP	TCA	1	5.1

To measure uptake activities, hepatocytes (1.0 x 10^6 cells/mL) in suspension were incubated in triplicate at 4°C and 37°C for 1 minute in Krebs-Henseleit buffer and marker substrate, at the final concentrations indicated. Uptake of substrate was measured by scintillation counter.