Subcellular Fractions

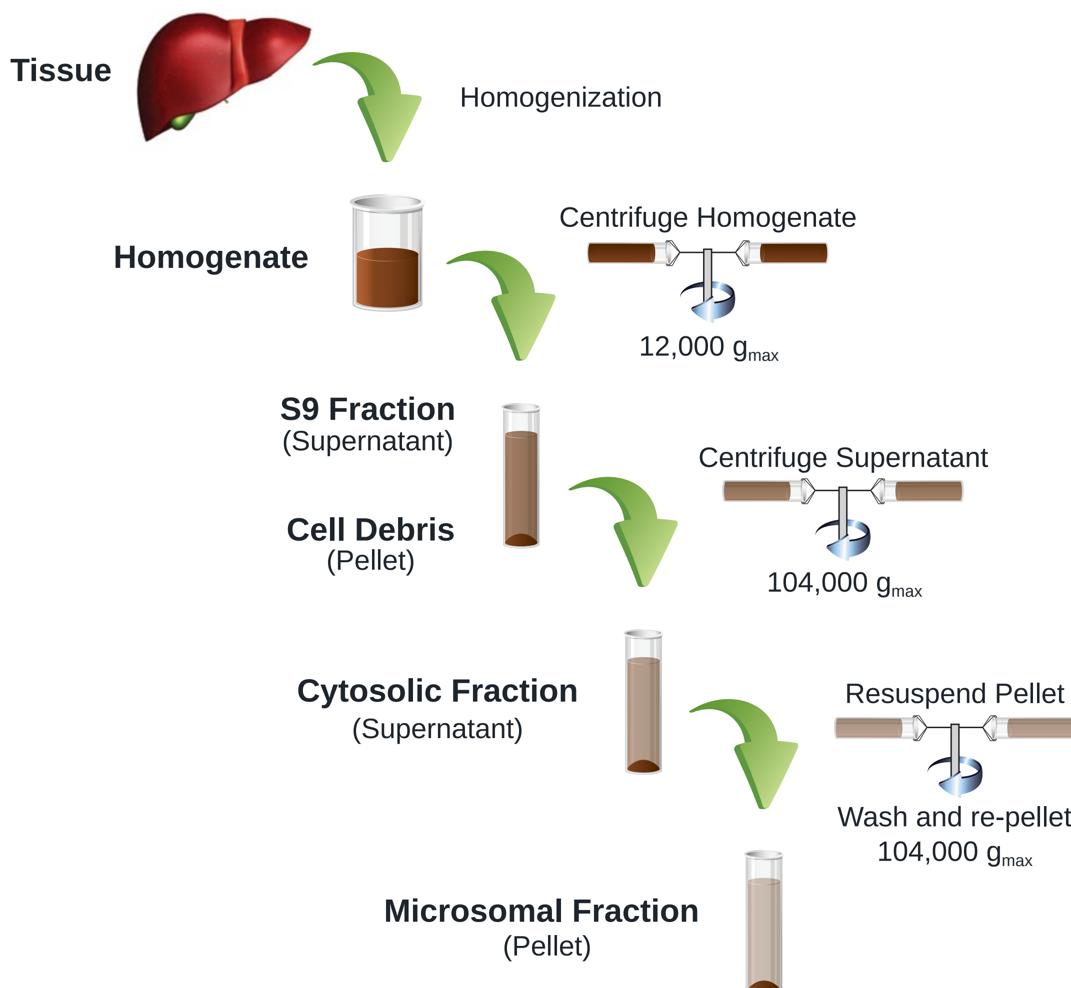
Sekisui XenoTech's subcellular fractions are widely used in drug discovery and preclinical drug development to evaluate species differences, similarities in metabolite formation by various species, metabolic stability, *in vitro* intrinsic clearance, reaction phenotyping (enzyme mapping) and enzyme inhibition.

Sekisui XenoTech's subcellular fractions have many unique advantages including large donor pools to minimize lot-to-lot variation and increase long-term lot availability, robust preparation procedures allowing for the production of matching S9 and microsomal donor pools, and unsurpassed quality control governed by the Study Director, the Study Manager and Sekisui XenoTech's Quality Control Unit.

Sekisui XenoTech has the widest selection of subcellular fractions on the market, with pool sizes up to 200 individual donors. Our XTreme 200 is the largest commercially available pool of human liver microsomes.

For specific information on all of our subcellular fractions, including preparation methods and characterization, please reference ***appendix pages 65-89***.

Isolation of Subcellular Fractions



To view detailed preparation methods, see ***appendix page 65***.

Human Liver Subcellular Fractions

Sekisui XenoTech offers a variety of different subcellular fraction products derived from human liver tissue:

- Homogenate
- Microsomes
- Genotyped microsomes
- S9 fraction
- Cytosol
- Mitochondria
- Lysosomes / Tritosomes

Sekisui XenoTech characterizes its subcellular fractions for CYP, FMO and UGT enzyme activities. Actual characterization varies by product and can be found starting on ***appendix page 77***.

Human Liver Homogenate

Sekisui XenoTech's human liver homogenate contains a total presentation of drug metabolizing enzymes and proteins. This fraction is ideal for metabolism identification (where enzymes responsible are not known), proteomic assays, enzymatic activity studies, etc.

Pooled Human Liver Homogenate

Product ID	*Description*	*Pool Size*	*Gender*	*Volume*
H0610.H	Pooled Human Liver Homogenate	20	Mixed	1.0 mL

**All human liver homogenate is supplied at a protein concentration of 20 mg/mL, in 50mM Tris-HCl containing 150mM KCl and 2mM EDTA.*

Pooled Human Liver Microsomes

Sekisui XenoTech features pooled microsome products that reflect enzymatic activity rates typically expressed in the general population. These pooled microsomes are prepared in large batches from at least 10, and up to, 200 donors. Sekisui XenoTech's pooled microsome products include mixed gender, gender-specific and CMV-free pools and are intended for use in *in vitro* studies of xenobiotic metabolism.

Pooled Human Liver Microsomes

Product ID	*Description*	*Pool Size*	*Gender*	*Volume*
H0610	Pooled Human Liver Microsomes	50	Mixed	0.5 mL
H0610-81	Pooled Human Liver Microsomes – Box of 81 Vials	50	Mixed	81 Vials, 0.5 mL/Vial
H0620	Pooled Human Liver Microsomes	50	Mixed	1.0 mL
H0630	Pooled Human Liver Microsomes	50	Mixed	5.0 mL
H0640	Pooled Human Liver Microsomes	50	Mixed	50 mL
H1000	Pooled Human Liver Microsomes	10	Male	0.5 mL
H1500	Pooled Human Liver Microsomes	10	Female	0.5 mL
H0604	CMV-Free Pooled Human Liver Microsomes	8	Mixed	0.5 mL

**All human liver microsomes are supplied at a protein concentration of 20 mg/mL in 250 mM sucrose.*

200-Pool Human Liver Microsomes

Product ID	*Description*	*Pool Size*	*Gender*	*Volume*
H2610	XTreme 200 Pooled Human Liver Microsomes	200	Mixed	0.5 mL
H2610-81	XTreme 200 Pooled Human Liver Microsomes – Box of 81 Vials	200	Mixed	81 Vials, 0.5 mL/Vial
H2620	XTreme 200 Pooled Human Liver Microsomes	200	Mixed	1.0 mL
H2630	XTreme 200 Pooled Human Liver Microsomes	200	Mixed	5.0 mL
H2640	XTreme 200 Pooled Human Liver Microsomes	200	Mixed	50 mL

**All human liver microsomes are supplied at a protein concentration of 20 mg/mL in 250 mM sucrose.*

Reaction Phenotyping Kit

Sekisui XenoTech's patented (# 5,478,723) Reaction Phenotyping Kit is designed to identify the human liver CYP and UGT enzymes(s) responsible for metabolizing a drug (or other xenobiotic), in order to predict pharmacokinetic variability, which can occur when a drug is metabolized by a polymorphically-expressed CYP or UGT enzyme. Reaction Phenotyping (enzyme mapping) also provides valuable information on the potential for drug-drug interactions. Samples in the kit are carefully selected to minimize correlations or outliers that can interfere with reliable results. Preparation procedures can be found starting on ***appendix page 75***.

Each kit contains:

- 16 individual samples of human liver microsomes
- 2 vials of pooled human liver microsomes
- Cytochrome P450 and UDP-glucuronosyltransferase

Correlation Analyses of Sample-to-Sample Variation in Cytochrome P450 Activities:
Evidence for independent variation of cytochrome P450 activities in a bank of human liver microsomes (Kit ver. 8)

CYP Enzyme	CYP2A6	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP2J2	CYP3A4/5 Testosterone	CYP3A4/5 Midazolam	CYP4A11	FMO
CYP1A2	0.525	0.198	0.536	0.504	0.255	0.017	0.125	0.024	0.171	0.254	0.322	0.014
CYP2A6		**0.622**	0.600	**0.607**	0.275	-0.251	0.111	0.028	0.436	0.531	0.096	-0.100
CYP2B6			0.340	0.038	**0.629**	-0.446	0.176	-0.118	0.390	0.493	-0.114	-0.213
CYP2C8				**0.667**	0.087	0.078	0.034	0.199	0.309	0.317	0.443	0.202
CYP2C9					0.009	0.240	0.123	0.539	0.575	0.558	0.602	0.102
CYP2C19						-0.151	-0.122	0.110	0.391	0.433	-0.058	-0.153
CYP2D6							-0.384	0.516	0.058	0.011	0.266	0.275
CYP2E1								-0.025	0.253	0.258	0.403	-0.308
CYP2J2									**0.644**	0.527	0.564	0.387
CYP3A4/5 Testosterone										**0.978**	0.265	0.075
CYP3A4/5 Midazolam											0.185	0.011
CYP4A11												0.197

**Data shown in bold-face type are statistically significant at p<0.01 for 16 human liver microsomal samples and 1 pool.*

Reaction Phenotyping Kit

Product ID	*Description*	*Gender*
H0500	Reaction Phenotyping Kit, Human Liver Microsomes (16 individuals plus 2 pooled vials)	Mixed

Human Liver Subcellular Fractions

Genotyped (Genetically-Defined) Human Liver Microsomes

Microsomes from many polymorphically-expressed enzymes including CYP2C9, CYP2C19, CYP2D6, CYP3A5, UGT1A1 and UGT1A9, are available in high (HA), moderate (MA) and no activity (NA) categories.

For general characterization information on genotyped microsomes, see ***appendix page 77***.

Genotyped Human Liver Microsomes

Product ID	*Description*	*Volume*
H2C9.HA	CYP2C9 – High Activity (*1/*1)	0.5 mL
H2C9.MA	CYP2C9 – Moderate Activity (*1/*2, *1/*3, *1/*5, *2/*2, *2/*3)	0.5 mL
H2C19.HA	CYP2C19 – High Activity (*1/*1)	0.5 mL
H2C19.MA	CYP2C19 – Moderate Activity (*1/*2, *1/*3, *1/*4, *1/*5)	0.5 mL
H2C19.NA	CYP2C19 – No Activity (*2/*2, *2/*3, etc.)	0.5 mL
H2D6.HA	CYP2D6 – High Activity (Activity score > 2.5)	0.5 mL
H2D6.MA	CYP2D6 – Moderate Activity (Activity Score = 0.5-2.5)	0.5 mL
H2D6.NA	CYP2D6 – No Activity (Activity Score = 0)	0.5 mL
H3A5.HA	CYP3A5 – High Activity (*1/*1)	0.5 mL
H3A5.MA	CYP3A5 – Moderate Activity (*1/*3)	0.5 mL
H3A5.NA	CYP3A5 – No Activity (*3/*3)	0.5 mL
HU1A1.HA	UGT1A1 – High Activity (*1/*1)	0.5 mL
HU1A1.MA	UGT1A1 – Moderate Activity (*1/*28)	0.5 mL
HU1A1.NA	UGT1A1 – No Activity (*28/*28)	0.5 mL
HU1A9.HA	UGT1A9 – High Activity (*1/*1)	0.5 mL
HU1A9.MA	UGT1A9 – Moderate Activity (*1/*3)	0.5 mL
HU1A9.NA	UGT1A9 – No Activity (*3/*3)	0.5 mL

**All genotyped human liver microsomes are supplied at a protein concentration of 20 mg/mL in 250 mM sucrose.*

Pooled Human Liver S9 Fraction

Sekisui XenoTech's S9 fraction (post-mitochondrial supernatant fraction) is a mixture of microsomes and cytosol. Human liver S9 fractions are best suited for studying phase I and phase II xenobiotic metabolism *in vitro*.

For specific characterization information, see ***appendix page 78***.

Pooled Human Liver S9 Fraction				
Product ID	***Description***	***Pool Size***	***Gender***	***Volume***
H2610.S9	XTreme 200 Pooled Human Liver S9 Fraction	200	Mixed	0.5 mL
H2620.S9	XTreme 200 Pooled Human Liver S9 Fraction	200	Mixed	1.0 mL
H2630.S9	XTreme 200 Pooled Human Liver S9 Fraction	200	Mixed	5.0 mL
H2640.S9	XTreme 200 Pooled Human Liver S9 Fraction	200	Mixed	50 mL
H0610.S9	Pooled Human Liver S9 Fraction	50	Mixed	0.5 mL
H0620.S9	Pooled Human Liver S9 Fraction	50	Mixed	1.0 mL
H0630.S9	Pooled Human Liver S9 Fraction	50	Mixed	5.0 mL
H0640.S9	Pooled Human Liver S9 Fraction	50	Mixed	50 mL
H1000.S9	Pooled Human Liver S9 Fraction	10	Male	1.0 mL
H1500.S9	Pooled Human Liver S9 Fraction	10	Female	1.0 mL

**S9 fractions are supplied at a concentration of 20 mg/mL, in 50 mM Tris-HCl, (pH 7.4 at 4°C) containing 150 mM KCl and 2 mM EDTA.*

Pooled Human Liver Cytosol

Sekisui XenoTech's human liver cytosol (the soluble portion of liver homogenate) contains most of the non-CYP enzymes and are best suited for *in vitro* xenobiotic metabolism studies.

For specific characterization information, see ***appendix page 78***.

Pooled Human Liver Cytosol				
Product ID	***Description***	***Pool Size***	***Gender***	***Volume***
H2610.C	XTreme 200 Pooled Human Liver Cytosol	200	Mixed	1.0 mL
H0610.C	Pooled Human Liver Cytosol	50	Mixed	1.0 mL
H1000.C	Pooled Human Liver Cytosol	10	Male	1.0 mL
H1500.C	Pooled Human Liver Cytosol	10	Female	1.0 mL

**Cytosol fractions are supplied at a concentration of 10 mg/mL in 50 mM Tris-HCl, (pH 7.4 at 4°C) containing 150 mM KCl and 2 mM EDTA.*

Liver Mitochondria

Sekisui XenoTech's human liver mitochondria contain monoamine oxidases A and B (MAO A and B), aldehyde dehydrogenases and other xenobiotic-metabolizing enzymes. These fractions are used to study compounds that are metabolized by mitochondrial enzymes.

Liver Mitochondria				
Product ID	***Description***	***Pool Size***	***Gender***	***Volume***
H0610.M	Pooled Human Liver Mitochondria	5	Mixed	0.5 mL

**Mitochondria fractions are supplied at a concentration of 20 mg/mL in 250 mM sucrose.*

Animal Liver Subcellular Fractions

Animal Liver Subcellular Fractions

Sekisui XenoTech offers a variety of liver subcellular fractions from toxicologically relevant species such as non-human primate, dog, rabbit, rat and mouse. These subcellular fractions can be used to evaluate metabolic stability, *in vitro* intrinsic clearance, reaction phenotyping and inhibition of CYP and UGT enzymes. Examples include drug safety studies in laboratory animals and studies of species, strain and gender differences in drug metabolism.

Sekisui XenoTech's animal liver microsomes are supplied in a 250 mM sucrose suspension buffer. S9 and cytosolic fractions are packaged in a suspension buffer containing 50 mM Tris-HCl (pH 7.4 at 4°C) containing 150 mM KCl and 2 mM EDTA.

For specific animal liver subcellular fraction characterization data, see ***appendix page 86***. Preparation procedures can be found starting on ***appendix page 65***.

Monkey – Rhesus

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
P1000	Pooled Rhesus Monkey Liver Microsomes	Male	0.5 mL	20 mg/mL
P1000.S9	Pooled Rhesus Monkey Liver S9 Fraction	Male	1.0 mL	20 mg/mL
P1000.C	Pooled Rhesus Monkey Liver Cytosol	Male	1.0 mL	10 mg/mL
P1500	Pooled Rhesus Monkey Liver Microsomes	Female	0.5 mL	20 mg/mL
P1500.S9	Pooled Rhesus Monkey Liver S9 Fraction	Female	1.0 mL	20 mg/mL
P1500.C	Pooled Rhesus Monkey Liver Cytosol	Female	1.0 mL	10 mg/mL

Monkey – Cynomolgus

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
P2000	Pooled Cynomolgus Monkey Liver Microsomes	Male	0.5 mL	20 mg/mL
P2000.S9	Pooled Cynomolgus Monkey Liver S9 Fraction	Male	1.0 mL	20 mg/mL
P2000.C	Pooled Cynomolgus Monkey Liver Cytosol	Male	1.0 mL	10 mg/mL
P2500	Pooled Cynomolgus Monkey Liver Microsomes	Female	0.5 mL	20 mg/mL
P2500.S9	Pooled Cynomolgus Monkey Liver S9 Fraction	Female	1.0 mL	20 mg/mL
P2500.C	Pooled Cynomolgus Monkey Liver Cytosol	Female	1.0 mL	10 mg/mL

Minipig – Sinclair

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
Z2000	Pooled Sinclair Minipig Liver Microsomes	Male	0.5 mL	20 mg/mL
Z2000.S9	Pooled Sinclair Minipig Liver S9 Fraction	Male	1.0 mL	20 mg/mL

Minipig – Yucatan

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
Z3000	Pooled Yucatan Minipig Liver Microsomes	Male	0.5 mL	20 mg/mL
Z3000.S9	Pooled Yucatan Minipig Liver S9 Fraction	Male	1.0 mL	20 mg/mL

Minipig – Gottingen

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
Z6000	Pooled Gottingen Minipig Liver Microsomes	Male	0.5 mL	20 mg/mL
Z6000.S9	Pooled Gottingen Minipig Liver S9 Fraction	Male	1.0 mL	20 mg/mL

Animal Liver Subcellular Fractions

Dog – Beagle

Product ID	Description	Gender	Volume	Concentration
D1000	Pooled Beagle Dog Liver Microsomes	Male	0.5 mL	20 mg/mL
D1000.S9	Pooled Beagle Dog Liver S9 Fraction	Male	1.0 mL	20 mg/mL
D1000.C	Pooled Beagle Dog Liver Cytosol	Male	1.0 mL	10 mg/mL
D1500	Pooled Beagle Dog Liver Microsomes	Female	0.5 mL	20 mg/mL
D1500.S9	Pooled Beagle Dog Liver S9 Fraction	Female	1.0 mL	20 mg/mL
D1500.C	Pooled Beagle Dog Liver Cytosol	Female	1.0 mL	10 mg/mL

Rabbit – New Zealand

Product ID	Description	Gender	Volume	Concentration
L1000	Pooled New Zealand Rabbit Liver Microsomes	Male	0.5 mL	20 mg/mL
L1000.S9	Pooled New Zealand Rabbit Liver S9 Fraction	Male	1.0 mL	20 mg/mL
L1000.C	Pooled New Zealand Rabbit Liver Cytosol	Male	1.0 mL	10 mg/mL
L1500	Pooled New Zealand Rabbit Liver Microsomes	Female	0.5 mL	20 mg/mL
L1500.S9	Pooled New Zealand Rabbit Liver S9 Fraction	Female	1.0 mL	20 mg/mL
L1500.C	Pooled New Zealand Rabbit Liver Cytosol	Female	1.0 mL	10 mg/mL

Guinea Pig – Hartley Albino

Product ID	Description	Gender	Volume	Concentration
G1000	Pooled Hartley Albino Guinea Pig Liver Microsomes	Male	0.5 mL	20 mg/mL
G1000.S9	Pooled Hartley Albino Guinea Pig Liver S9 Fraction	Male	1.0 mL	20 mg/mL
G1000.C	Pooled Hartley Albino Guinea Pig Liver Cytosol	Male	1.0 mL	10 mg/mL

Rat – IGS Sprague-Dawley

Product ID	Description	Gender	Volume	Concentration
R1000	Pooled IGS Sprague-Dawley Rat Liver Microsomes	Male	0.5 mL	20 mg/mL
R1000.S9	Pooled IGS Sprague-Dawley Rat Liver S9 Fraction	Male	1.0 mL	20 mg/mL
R1000.C	Pooled IGS Sprague-Dawley Rat Liver Cytosol	Male	1.0 mL	10 mg/mL
R1500	Pooled IGS Sprague-Dawley Rat Liver Microsomes	Female	0.5 mL	20 mg/mL
R1500.S9	Pooled IGS Sprague-Dawley Rat Liver S9 Fraction	Female	1.0 mL	20 mg/mL
R1500.C	Pooled IGS Sprague-Dawley Rat Liver Cytosol	Female	1.0 mL	10 mg/mL

Rat – Fischer 344

Product ID	Description	Gender	Volume	Concentration
R2000	Pooled Fischer 344 Rat Liver Microsomes	Male	0.5 mL	20 mg/mL
R2000.S9	Pooled Fischer 344 Rat Liver S9 Fraction	Male	1.0 mL	20 mg/mL
R2000.C	Pooled Fischer 344 Rat Liver Cytosol	Male	1.0 mL	10 mg/mL
R2500	Pooled Fischer 344 Rat Liver Microsomes	Female	0.5 mL	20 mg/mL
R2500.S9	Pooled Fischer 344 Rat Liver S9 Fraction	Female	1.0 mL	20 mg/mL
R2500.C	Pooled Fischer 344 Rat Liver Cytosol	Female	1.0 mL	10 mg/mL

Animal Liver Subcellular Fractions *(cont.)*

Rat – Wistar

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
R3000	Pooled Wistar Rat Liver Microsomes	Male	0.5 mL	20 mg/mL
R3000.S9	Pooled Wistar Rat Liver S9 Fraction	Male	1.0 mL	20 mg/mL
R3000.C	Pooled Wistar Rat Liver Cytosol	Male	1.0 mL	10 mg/mL
R3500	Pooled Wistar Rat Liver Microsomes	Female	0.5 mL	20 mg/mL
R3500.S9	Pooled Wistar Rat Liver S9 Fraction	Female	1.0 mL	20 mg/mL
R3500.C	Pooled Wistar Rat Liver Cytosol	Female	1.0 mL	10 mg/mL

Rat – Wistar Han

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
R6000	Pooled Wistar Han Rat Liver Microsomes	Male	0.5 mL	20 mg/mL
R6000.S9	Pooled Wistar Han Rat Liver S9 Fraction	Male	1.0 mL	20 mg/mL
R6000.C	Pooled Wistar Han Rat Liver Cytosol	Male	1.0 mL	10 mg/mL

Hamster – Golden Syrian

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
S1000	Pooled Golden Syrian Hamster Liver Microsomes	Male	0.5 mL	20 mg/mL
S1000.S9	Pooled Golden Syrian Hamster Liver S9 Fraction	Male	1.0 mL	20 mg/mL
S1000.C	Pooled Golden Syrian Hamster Liver Cytosol	Male	1.0 mL	10 mg/mL

Mouse – CD-1

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
M1000	Pooled CD-1 Mouse Liver Microsomes	Male	0.5 mL	20 mg/mL
M1000.S9	Pooled CD-1 Mouse Liver S9 Fraction	Male	1.0 mL	20 mg/mL
M1000.C	Pooled CD-1 Mouse Liver Cytosol	Male	1.0 mL	10 mg/mL
M1500	Pooled CD-1 Mouse Liver Microsomes	Female	0.5 mL	20 mg/mL
M1500.S9	Pooled CD-1 Mouse Liver S9 Fraction	Female	1.0 mL	20 mg/mL
M1500.C	Pooled CD-1 Mouse Liver Cytosol	Female	1.0 mL	10 mg/mL

Mouse – B6C3F1

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
M2000	Pooled B6C3F1 Mouse Liver Microsomes	Male	0.5 mL	20 mg/mL
M2000.S9	Pooled B6C3F1 Mouse Liver S9 Fraction	Male	1.0 mL	20 mg/mL
M2000.C	Pooled B6C3F1 Mouse Liver Cytosol	Male	1.0 mL	10 mg/mL

Mouse – BALB/c

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
M3000	Pooled BALB/c Mouse Liver Microsomes	Male	0.5 mL	20 mg/mL
M3000.S9	Pooled BALB/c Mouse Liver S9 Fraction	Male	1.0 mL	20 mg/mL
M3000.C	Pooled BALB/c Mouse Liver Cytosol	Male	1.0 mL	10 mg/mL

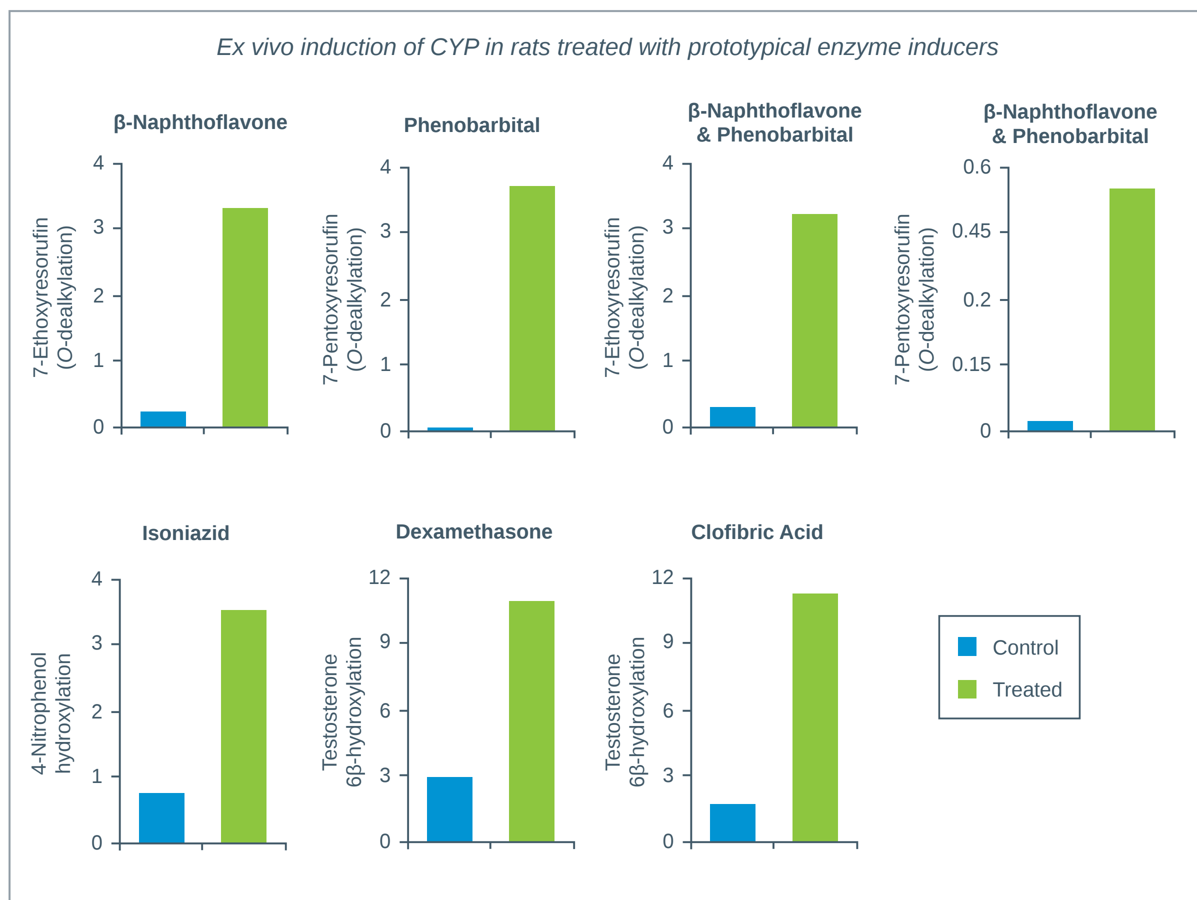
Mouse – C57BL/6

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
M5000	Pooled C57BL/6 Mouse Liver Microsomes	Male	0.5 mL	20 mg/mL
M5000.S9	Pooled C57BL/6 Mouse Liver S9 Fraction	Male	1.0 mL	20 mg/mL
M5000.C	Pooled C57BL/6 Mouse Liver Cytosol	Male	1.0 mL	10 mg/mL

Animal Liver Subcellular Fractions

Treated Animal Liver Subcellular Fractions

Treatment of animals with various xenobiotics may cause a marked induction of liver microsomal CYP levels, which, in chronic studies may be associated with liver and/or thyroid tumor formation. Sekisui XenoTech offers treated animal liver subcellular fractions from non-human primate, dog and rat. These fractions are best suited as positive controls for *ex vivo* enzyme induction studies.



** Rates are expressed as nmol/mg protein/min.*

Treated Animal Liver Subcellular Fractions *(cont.)*

The following tables represent Sekisui XenoTech's current treated animal liver subcellular fraction products.

Treated Monkey – Cynomolgus

Product ID	*Description*	*Gender*	*Volume*	*Concentration*	*Induced CYP Control*
P2073	Saline-treated Cynomolgus Monkey Microsomes	Male	0.5 mL	10 mg/mL	Vehicle Control
P2078	Phenobarbital-treated Cynomolgus Monkey Microsomes	Male	0.5 mL	10 mg/mL	CYP2A, CYP2B
P2083	BNF-treated Cynomolgus Monkey Microsomes	Male	0.5 mL	10 mg/mL	CYP1A
P2085	Pyrazole-treated Cynomolgus Monkey Microsomes	Male	0.5 mL	10 mg/mL	CYP2E
P2095	Rifampin-treated Cynomolgus Monkey Microsomes	Male	0.5 mL	10 mg/mL	CYP3A
P2096	Omeprazole-treated Cynomolgus Monkey Microsomes	Male	0.5 mL	10 mg/mL	CYP1A
P2573	Saline-treated Cynomolgus Monkey Microsomes	Female	0.5 mL	10 mg/mL	Vehicle Control
P2578	Phenobarbital-treated Cynomolgus Monkey Microsomes	Female	0.5 mL	10 mg/mL	CYP2A, CYP2B
P2583	BNF-treated Cynomolgus Monkey Microsomes	Female	0.5 mL	10 mg/mL	CYP1A
P2585	Pyrazole-treated Cynomolgus Monkey Microsomes	Female	0.5 mL	10 mg/mL	CYP2E
P2595	Rifampin-treated Cynomolgus Monkey Microsomes	Female	0.5 mL	10 mg/mL	CYP3A
P2596	Omeprazole-treated Cynomolgus Monkey Microsomes	Female	0.5 mL	10 mg/mL	CYP1A

Treated Dog – Beagle

Product ID	*Description*	*Gender*	*Volume*	*Concentration*	*Induced CYP Control*
D1063	Clofibric acid-treated Beagle Dog Microsomes	Male	0.5 mL	20 mg/mL	CYP4A
D1073	Saline-treated Beagle Dog Microsomes	Male	0.5 mL	20 mg/mL	Vehicle Control
D1078	Phenobarbital-treated Beagle Dog Microsomes	Male	0.5 mL	20 mg/mL	CYP2B
D1083	BNF-treated Beagle Dog Microsomes	Male	0.5 mL	20 mg/mL	CYP1A
D1095	Rifampin-treated Beagle Dog Microsomes	Male	0.5 mL	20 mg/mL	CYP3A
D1098	Corn Oil-treated Beagle Dog Microsomes	Male	0.5 mL	20 mg/mL	Vehicle Control

Treated Rat – IGS Sprague-Dawley

Product ID	*Description*	*Gender*	*Volume*	*Concentration*	*Induced CYP Control*
R1063	Clofibric Acid-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	CYP4A
R1073	Saline-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	Vehicle Control
R1078	Phenobarbital-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	CYP2B
R1081	BNF and Phenobarbital-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	CYP1A, CYP2B
R1081.S9	S9 Fraction from and Phenobarbital-treated Sprague-Dawley Rat Microsomes	Male	1.0 mL	20 mg/mL	CYP1A, CYP2B
R1083	BNF-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	CYP1A
R1088	Isoniazid-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	CYP2E
R1093	Dexamethasone-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	CYP3A
R1098	Corn Oil-treated Sprague-Dawley Rat Microsomes	Male	0.5 mL	20 mg/mL	Vehicle Control

Lysosomes / Tritosomes

Lysosomes are a site of catabolism of diverse biological components of the cell. Degradative enzymes present in the organelle can be used to determine efficacy of nucleic acid-based drugs or antibody drug conjugates.

Human Liver Lysosomes and Rat Liver Tritosomes

Lysosomes/tritosomes can be used as an in vitro diagnostic tool to conveniently, cost-effectively and quickly evaluate potential changes in lysosomal stability due to targeted modifications of the biopharmaceutical / macromolecule during development. The data can help narrow and direct development tracks of biopharmaceuticals such as those interested in ADCs, siRNA/RNAi technologies, immunotherapies, biodegradable copolymers and nanoparticles as delivery mechanisms; cosmetics using microparticles; etc.

Rat liver tritosomes are hepatic lysosomes that have been loaded with Tyloxapol (Triton WR 1339), a non-ionic surfactant, which is taken up by hepatocytes through endocytosis and is trafficked to lysosomal compartments. Tyloxapol containing lysosomes exhibit decreased density and can be efficiently separated from other cellular organelles that have density overlapping the native lysosomes. Tritosomes are used to study lysosomal composition, function, and disease. More recently they have become a powerful reagent to assess the in vitro stability and release of small molecular moieties conjugated to nanoparticles, polymers and antibodies that enter cells through the endosome-lysosome pathway.

For more information on tritosome assays, please see ***appendix page 87.***

Features and Benefits:

- Highly purified
- Characterized for lysosome specific enzymatic activity
- Less complex than in vivo models
- More representative than using individually expressed/purified enzymes

Lysosomes and Tritosomes

Product ID	***Description***	***Pool Size***	***Gender***	***Volume***
H0610.L	Pooled Human Liver Tritosomes	4	Mixed	0.25 mL
R0610.LT	Pooled IGS Sprague-Dawley Rat Liver Tritosomes	60	Mixed	0.25 mL

Sekisui XenoTech's 10x catabolism buffer has been formulated and optimized to extract the most in vitro catabolic performance from isolated human lysosomes and rat tritosomes. Please note: *although DTT is present in the buffer, it can easily be removed.*

Lysosome/Tritosome Media

Product ID	***Description***	***Volume***
K5200	10x Catabolic Buffer	1 mL

Contact us to learn more at **www.xenotech.com** or call us at **913.GET.P450**

Custom Products

Sekisui XenoTech's custom products division has over 15 years of experience preparing hepatocytes and subcellular fractions from a variety of species. All Sekisui XenoTech custom product preparations are managed by a dedicated Senior Production Scientist to ensure the highest quality for each custom request. We use custom-designed preparation methods and offer more than 40 characterization assays validated by LC-MS/MS for your custom needs.

We routinely prepare custom batches of hepatic, intestinal, pulmonary and renal subcellular fractions in accordance with client specifications. As a result from these custom preparations, we have a surplus inventory of products remaining; to view a complete list of surplus inventory, please visit: **xenotech.com/Products/Custom-Products**.

The list provided below shows examples of products Sekisui XenoTech has prepared in the past and is not a complete list of Sekisui XenoTech's capabilities for custom product preparations.

Available Characterization

Content

Total P450 content by spectral analysis
Cytochrome b_5 content by spectral analysis
NADPH-cytochrome *c* reductase activity

Cytochrome P450 Activity

7-Benzoxyresorufin *O*-dealkylation
7-Ethoxyresorufin *O*-dealkylation
7-Methoxyresorufin *O*-dealkylation
7-Pentoxyresorufin *O*-dealkylation
7-Ethoxycoumarin *O*-dealkylation
Phenactin *O*-dealkylation
Coumarin 7-hydroxylation
Bupropion hydroxylation
Amodiaquine *N*-dealkylation
Diclofenac 4′-hydroxylation
S-Mephenytoin 4′-hydroxylation
Dextromethorphan *O*-demethylation
Chlorzoxazone *6*-hydroxylation
Ebastine hydroxylation
Testosterone 2α-hydroxylation
Testosterone 2β-hydroxylation
Testosterone 6β-hydroxylation
Testosterone 7α-hydroxylation
Testosterone 15α-hydroxylation
Testosterone 16α-hydroxylation
Testosterone 16β-hydroxylation
Midazolam 1′-hydroxylation
Lauric acid 12-hydroxylation
4-Nitrophenol hydroxylation
Tolbutamide hydroxylation

UDP-Glucuronosyltransferase Activity

4-Methylumbelliferone glucuronidation
17β-Estradiol 3-glucuronidation
Trifluoperazine glucuronidation
1-Naphthol glucuronidation
Propofol glucuronidation
Morphine 3-glucuronidation
Chenodeoxycholic acid 24-glucuronidation
Testosterone 17-glucuronidation
7-Hydroxycoumarin glucuronidation
7-Hydroxycoumarin sulfonation

Monoamine Oxidase Activity

5-Hydroxytryptamine oxidation
4-(Dimethylamino)benzylamine oxidation

Carboxylesterase Activity

Clopidogrel hydrolysis
Methylprednisolone 21-hemisuccinate hydrolysis

Glutathione *S*-Transferase Activity

1 Chloro-2,4-dinitrobenzene-glutathione conjugation

N-Acetyltransferase Activity

p-Aminobenzoic acid acetylation
Sulfamethazine acetylation
Dapsone acetylation

Aldehyde Oxidase

Phthalazine oxidation

Lysosomal Enzymes

Acid Phosphatase
RNase
Cathepsin B

Marker Substrate Enzymatic Fold Induction

CYP1A2
CYP2B6
CYP3A4

mRNA Fold Induction

CYP1A2
CYP2B6
CYP3A4

Uptake Transporter

OATP1B1
OATP1B3
OCT1
NTCP

Typical custom preparations vary in time to completion. This variation is caused by a number of factors including how long it takes to receive the specific tissue requested and the number of characterization assays requested. A typical custom product preparation usually takes between 4-12 weeks.

Appendix: Subcellular Fractions

Liver Subcellular Fractions

Preparation of Liver Homogenate

Tissue is homogenized in homogenization buffer[1]. Approximately, 2 to 3 mL of homogenization buffer is used per gram of wet tissue weight to produce a 25-33% homogenate. The homogenate is subjected to centrifugation at 600-700 g_{max} for 20 ± 1 minutes. The homogenate is stored at or below -70°C.

Preparation of Liver Microsomes, S9, and Cytosol

Tissue is homogenized in homogenization buffer[1]. Typically, 2 to 3 mL of homogenization buffer is used per gram of wet tissue weight to produce a 25-33% homogenate. The homogenate is subjected to centrifugation at 12,000-13,000 g_{max} for 20 ± 1 minutes at 0-8°C to prepare a post-mitochondrial supernatant (S9) fraction. The post-mitochondrial supernatant (S9) fraction is subjected to ultracentrifugation at 104,000-109,000 g_{max} for 60 ± 5 minutes at 0-8°C to prepare the cytosolic supernatant fraction. The remaining microsomal pellet is then resuspended in wash buffer[2] and subjected to ultracentrifugation at 104,000-109,000 g_{max} for 60 ± 5 minutes at 0-8°C. The supernatant is discarded leaving the washed microsomal pellet. Microsomes are resuspended in 250 mM sucrose and stored at or below -70°C.

Preparation of Liver Mitochondria

Tissue is homogenized in homogenization buffer[1]. Approximately, 2 to 3 mL of homogenization buffer is used per gram of wet tissue weight to produce a 25-33% homogenate. The homogenate is subjected to centrifugation at 400-500 g_{max} for 15 ± 1 minutes at 0-8°C to remove cell debris and nuclei. The supernatant fraction is subjected to centrifugation at 12,000-13,000 g_{max} for 15 ± 1 minutes at 0-8°C to separate the mitochondria (pellet) from the S9 fraction (supernatant). The initial crude mitochondrial pellet is resuspended in wash buffer[2] and re-isolated by centrifugation at 7,500-8,000 g_{max} for 15 ± 1 minutes at 0-8°C. Resuspension and re-isolation of the mitochondrial pellet is repeated. After the final wash, the mitochondrial pellet is resuspended in 250 mM sucrose and stored at or below -70°C.

[1]Homogenization Buffer
50 mM Tris-HCl, pH 7.4 at 4°C
150 mM Potassium chloride
2 mM EDTA, pH 7.4

[2]Wash Buffer
150 mM Potassium chloride
10 mM EDTA, pH 7.4

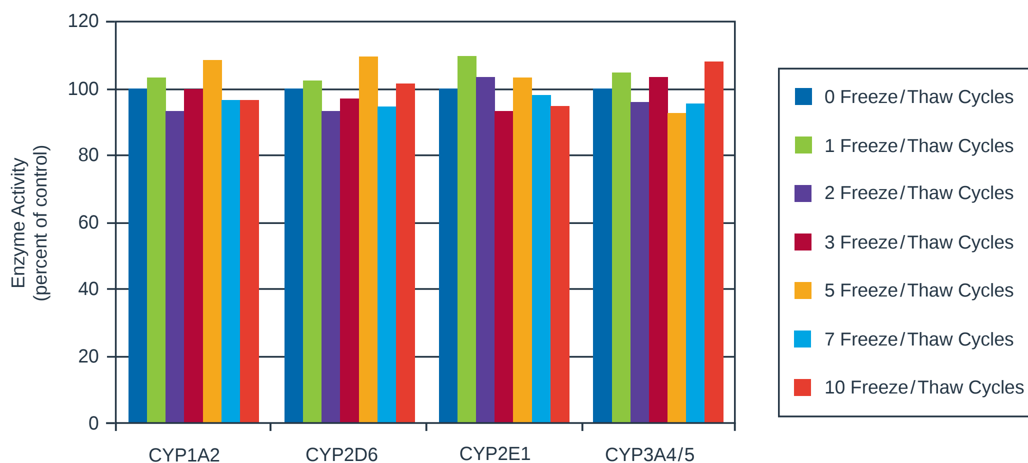
Comparison of XTreme 200 Pool vs. Pool of 50 Human Liver Microsomes

	Pool of 200 Donors	*Pool of 50 Donors*
CYP Activity	Better represents average American population	Slightly higher CYP activity than general population
Supply	>50 L of microsomes from same donor pool	>15 L of microsomes from same donor pool
Characterization	Includes enzymatic rates at multiple times K_m for all major CYPs, UGTs and FMO; also includes kinetic constants for major CYPs (K_m, V_{max}, CL_{int})	Includes enzymatic rates at multiple times K_m for all major CYPs, UGTs and FMO
Donor Representation	Each donor is equally represented according to tissue weight; equal number of males and females	Each donor is not equally represented, nor are males and females equally represented
Individual Liver Samples	Each liver sample is not prescreened for enzymatic viability of all CYPs	Each individual liver sample is verified to have optimal enzymatic activities, prior to pooling

Appendix: Subcellular Fractions

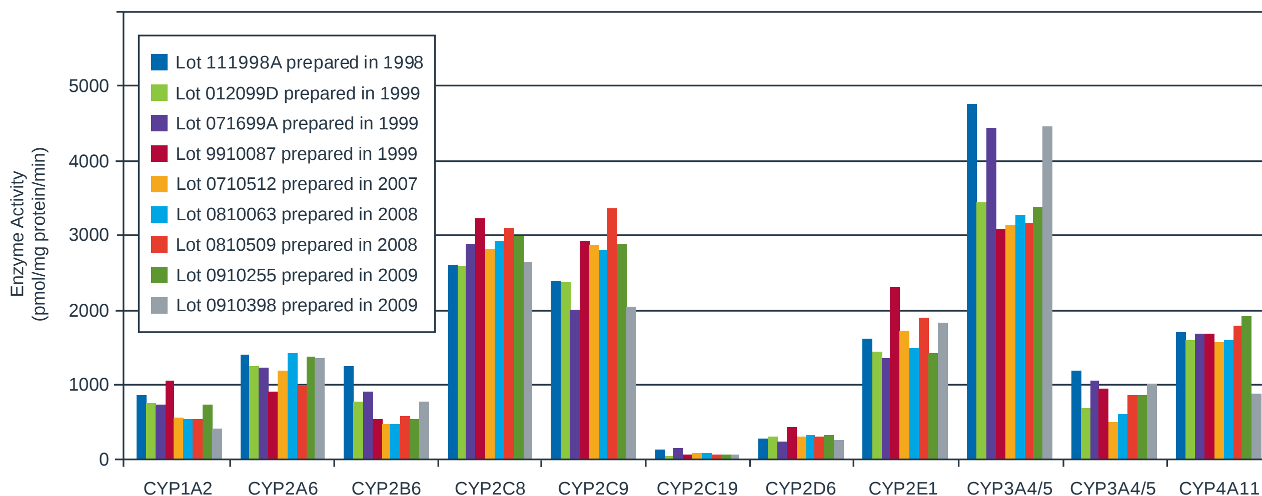
Human Liver Microsomes

Effect of repetitive freezing and thawing of pooled human liver microsomes on CYP1A2, CYP2D6, CYP2E1 and CYP3A4/5 enzyme activities

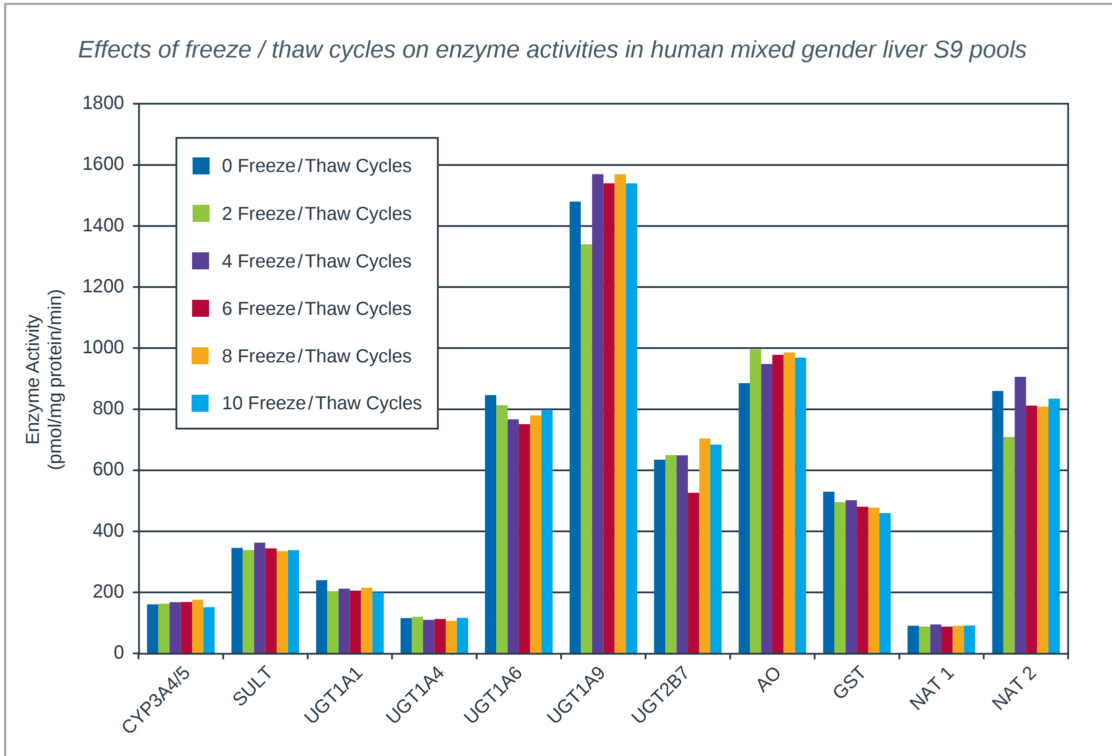


CYP450 Stability in Pooled Human Liver Microsomes

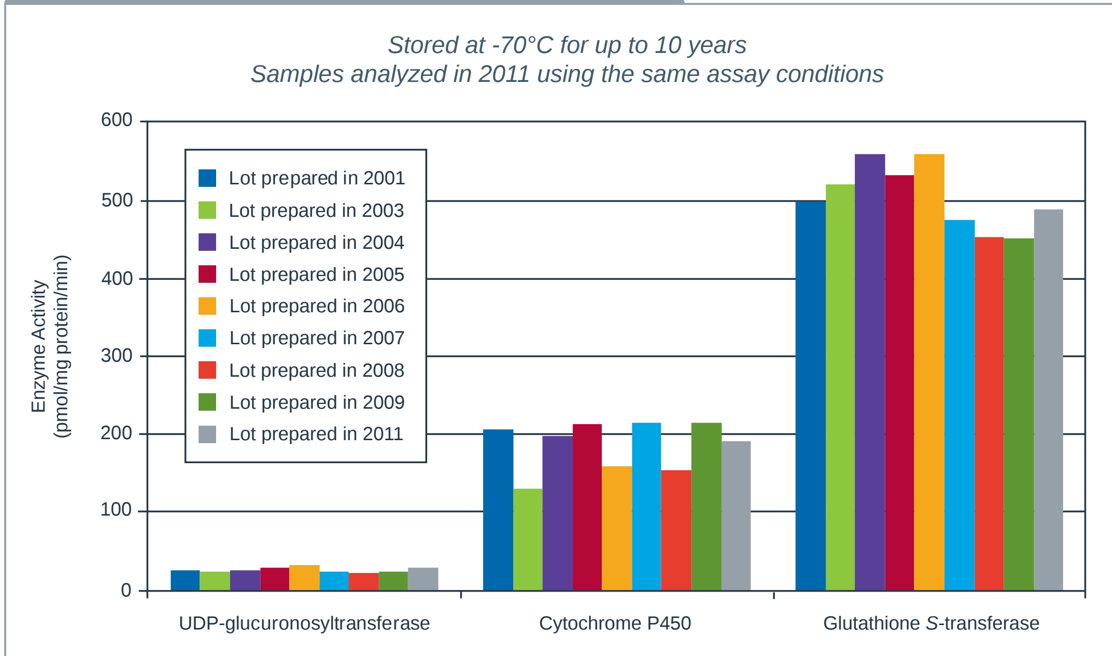
Stored at -70°C for up to 12 years
Samples analyzed in 2010 using the same assay conditions



Human Liver S9 Pools



Enzyme Stability in Pooled Human Liver S9 Fractions



Appendix: Subcellular Fractions

Microsome and S9 Fraction Enzyme Activity Characterization Assays

Cytochrome P450 Content by Spectral Analysis

The concentration of hemoglobin, cytochrome b_5 and cytochrome P450 is determined with a dual beam spectrophotometer.

The concentration of cytochrome b_5 and cytochrome P450 in subcellular fractions is often determined by the original method of Omura and Sato. However, this method of determining the concentration of cytochrome P450 is influenced by the presence of contaminating hemoglobin, which occurs, for example, when microsomes are prepared from frozen and/or non-perfused liver samples. Therefore, microsomes prepared from frozen liver samples may be analyzed by the method of Matsubara *et al.*, which has been modified so that the concentration of hemoglobin, cytochrome b_5 and cytochrome P450 can all be measured in the same sample. This modification is described below.

1. For use with 1-mL cuvettes, prepare at least 2.0-2.5 mL of microsomes typically diluted to 1.0 mg/mL in potassium phosphate buffer (100 mM, pH 7.4). Keep the samples at 2-8°C (*i.e.*, on ice). A wide range of protein concentrations (0.1-5.0 mg/mL) can be accommodated. Note: For S9 fractions, increase the normal protein concentration by a factor of 3 or 4. (*i.e.*, 3-4 mg/mL working concentration).
2. Add 1.0 mL of the diluted microsomes to each of the 1-mL sample and reference cuvettes. (If 0.5-mL or 3-mL cuvettes are used, the volume of diluted microsomes added to the cuvettes should be decreased or increased accordingly).
3. Record a baseline of equal light absorbance between 400 nm and 500 nm.
4. Saturate the contents of the sample cuvette with 30-40 bubbles of carbon monoxide. The flow rate should be approximately 2 bubbles/second; hence, the procedure should take 15-20 seconds. Measure the absorbance from 400 nm to 500 nm. The concentration of hemoglobin is determined from the absorbance difference between 420 nm (peak) and 405 nm (trough), based on an extinction coefficient of 104 $mM^{-1}cm^{-1}$.
5. After recording the hemoglobin spectrum, saturate the contents of the reference cuvette with 30-40 bubbles of carbon monoxide. To reduce cytochrome b_5, add 5 µL of 20 mM NADH (β-Nicotinamide Adenine Dinucleotide, reduced form) to the reference cuvette. Record the spectrum from 500 nm to 400 nm. The concentration of cytochrome b_5 is determined from the absorbance difference between 425 nm (peak) and 410 nm (trough), based on an extinction coefficient of 185 $mM^{-1}cm^{-1}$.
6. After recording the b_5 spectrum, add a few grains of solid sodium dithionite to the sample cuvette. The amount of dithionite is not weighed but is sufficient to cover the tip of a small spatula. The actual weight is typically between 0.2 and 1.0 mg. Saturate the contents of the sample cuvette with 30-40 bubbles of carbon monoxide.
7. Record the CO-difference spectrum of reduced microsomes between 400 nm and 500 nm. The spectrum of carboxyferrocytochrome P450 versus ferricytochrome P450 is characterized by an absorbance peak at 450 nm, an isosbestic point at 490 nm, a trough from 405 nm to 410 nm and possibly with a shoulder or second absorbance peak at 420 nm. The concentration of cytochrome P450 is determined from the absorbance difference between 450 nm (peak) and 490 nm (isosbestic point), based on an extinction coefficient of 110 $mM^{-1}cm^{-1}$.

Calculation of Cytochrome P450 Concentration

The concentration of cytochrome P450 (nmol/mL) in the sample cuvette is determined from the absorbance difference between 450 nm (peak) and 490 nm (isosbestic point) based on an extinction coefficient of 110 $mM^{-1}cm^{-1}$ (which is the same as 0.110 $\mu M^{-1}cm^{-1}$).

$$[\text{Cytochrome P450}] = A_{450} - A_{490} \times \frac{1}{\text{lightpath (cm)}} \times \frac{1}{0.110\ \mu M^{-1}cm^{-1}}$$

$A_{450} - A_{490}$ = Absorbance difference between 450 nm (peak and 490 nm (isosbestic point)
Lightpath = Distance that light travels through the cuvettes
0.110 $\mu M^{-1}cm^{-1}$ = Extinction coefficient of carboxyferrocytochrome P450 versus ferricytochrome P450

This calculation determines the concentration of cytochrome P450 in the sample cuvette (in units of nmol/mL). The specific content of cytochrome P450 (nmol cytochrome P450/mg microsomal protein) is calculated by dividing the concentration of cytochrome P450 (nmol/mL) by the concentration of microsomal protein (mg/mL) in the sample cuvette. If the samples were diluted to a protein concentration of 1.0 mg/mL, the concentration of cytochrome P450 (nmol/mL) in the sample cuvette will equal the specific content of cytochrome P450 (in units of nmol/mg protein). To calculate the concentration of cytochrome P450 in the original (undiluted) sample, this value must be multiplied by the dilution factor.

In cuvettes with a 1 cm lightpath, a sample containing 1 µM cytochrome P450 would produce an absorbance value of 0.110 between 450 nm and 490 nm.

NADPH-Cytochrome *c* Reductase Assay

Incubations are typically conducted in a 96-well microtiter plate with a final incubation volume of 250 µL (typically 150 µL cytochrome *c* working solution, 50 µL biological sample and 50 µL β-NADPH). A 96-well plate is set up containing two or more oxidized cytochrome *c* standards and reduced cytochrome *c* standards, as well as test sample wells, all of which contain liver microsomal samples and 50 µM cytochrome *c* (*i.e.*, 150 µL of 83.3 µM). Each well should contain between 1.0 and 50 µg protein (typically 50 µL of 62.5 µg/mL stock; 3.125 µg/well) and 50 µM cytochrome *c*. The volume in wells containing oxidized cytochrome *c* standard is adjusted to 250 µL by the addition of high purity water whereas the volume in wells containing reduced cytochrome *c* standard is adjusted to 250 µL by the addition of sodium dithionite (*e.g.*, 50 µL of 250 mg/mL).

Note: The wells containing oxidized and reduced cytochrome c standards must contain a final volume equal to that of the test sample incubations. The entire plate is pre-incubated (typically directly in the microtiter plate reader) at 30 ± 1°C for 5.0 ± 0.5 minutes. Reactions (test samples only) are started by the addition of NADPH to each well (typically 50 µL of 500 µM stock; 100 µM final concentration).

The rate of reduction of cytochrome *c* (at 30 ± 1°C) is determined by measuring the rate of change in the optical density (OD) at 550 nm in the linear portion of the kinetic curve (typically between 20 seconds and 2 minutes following the addition of β-NADPH).

Cytochrome P450 Activity Characterization – LC/MS/MS Method

Liver microsomes[1] (*e.g.*, 0.05 mg/mL) are incubated in triplicate at 37 ± 1°C in a 200 µL incubation mixture (final volume) containing potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), and EDTA (1 mM) and marker substrate, at the final concentration indicated. Reactions are started by the addition of a NADPH-regenerating system [NADP (1 mM), glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (1 U/mL)], and are stopped after 10 minutes by the addition of 0.175 mL acetonitrile containing an internal standard. If possible, a selected microsomal sample is incubated for approximately half and twice the regular

Appendix: Subcellular Fractions

Cytochrome P450 Activity Characterization – LC/MS/MS Method *(cont.)*

incubation period (to verify metabolite formation is proportional to incubation time) and at approximately half and twice the regular protein concentration (to ensure that metabolite formation is proportional to enzyme concentration). Precipitated protein is removed by centrifugation (920 g_{max} for 10 minutes at 10°C) and supernatant fractions are analyzed for metabolite formation by LC/MS/MS.

Zero-time incubations serve as blanks, and blanks spiked with various concentrations of known metabolites serve as standards. Deuterated metabolites serve as internal standards.

[1]S9 fractions should be incubated at 0.2 mg/mL.

Typical Incubation Conditions and Substrate Concentrations for Cytochrome P450 Characterization Assays (LC/MS/MS)

CYP	*Substrate*	*Substrate Concentration[2] (µM)*	*Substrate Solvent (final % v/v)*	*Metabolite Standards*
Various	7-Ethoxycoumarin	500	Methanol (0.5%)	2-400 ng/mL 7-Hydroxycoumarin
CYP1A2	Phenacetin	80	Methanol (1%)	1-200 ng/mL Acetaminophen
CYP2A6	Coumarin	50	Methanol (0.5%)	0.3-60 ng/mL 7-Hydroxycoumarin
CYP2B6	Bupropion	500	High purity water	2-400 ng/mL Hydroxybupropion
CYP2C8	Amodiaquine	20	High purity water	4-800 ng/mL N-Desethylamodiaquine
CYP2C9	Diclofenac	100	High purity water	3-600 ng/mL 4′-Hydroxydiclofenac
CYP2C19	*S*-Mephenytoin	400	Methanol (1%)	0.5-100 ng/mL 4′-Hydroxymephenytoin
CYP2D6	Dextromethorphan	80	High purity water	0.5-100 ng/mL Dextrorphan
CYP2E1	Chlorzoxazone	500	Potassium hydroxide (5%)	2-400 ng/mL 6-Hydroxychlorzoxazone
CYP2J2	Ebastine	30	Methanol (1%)	5-1000 ng/mL Hydroxyebastine
CYP3A4/5	Testosterone	250	Methanol/Acetonitrile (1%/0.5%)	10-2000 ng/mL 6β-Hydroxytestosterone
CYP3A4/5	Midazolam	30	Methanol (1%)	1-200 ng/mL 1-Hydroxymidazolam
CYP4A11	Lauric Acid	100	Na_2CO_3/Methanol (102 µM/0.94%)	2-400 ng/mL 12-Hydroxylauric acid

[2]The substrate concentration listed is near the 10x K_m for the reaction, and has been shown to be appropriate for metabolite formation.

Internal Standards for Cytochrome P450 Characterization Assays

Substrate	*Internal Standard*
7-Ethoxycoumarin	7-Hydroxycoumarin-d5
Phenacetin	Acetaminophen-d4
Coumarin	7-Hydroxycoumarin-d5
Bupropion	Hydroxybupropion-d6
Amodiaquine	*N*-Desethylamodiaquine-d5
Diclofenac	4′-Hydroxydiclofenac-d4
S-Mephenytoin	4′-Hydroxymephenytoin-d3
Dextromethorphan	Dextrorphan-d3
Chlorzoxazone	6-Hydroxychlorzoxazone-d2
Ebastine	Hydroxyebastine-d5
Testosterone	6β-Hydroxytestosterone-d2
Midazolam	α-Hydroxymidazolam-d3
Lauric Acid	12-Hydroxylauric acid-d20

Benzydamine *N*-oxidation Assay

Liver microsomes (1.0 mg/mL) are incubated at 37±1°C in 0.5 mL incubation mixtures containing tricine (49 mM, pH 8.5) and benzydamine (500 µM), at the final concentrations indicated. Reactions are started by addition of a NADPH-generating system [NADP (1 mM), glucose-6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (1 Unit/mL)], and are stopped after 10 minutes by the addition of 0.5 mL of paroxetine (internal standard) in methanol. Precipitated protein is removed by centrifugation (920 × *g* for 10 minutes at 10°C) and supernatant fractions are analyzed for metabolite formation by LC/MS/MS. Zero-time incubations serve as blanks, and blanks spiked with the metabolite standard solution (0.24 - 48 µM benzydamine N-oxide maleate, final concentrations) serve as metabolite standards.

UDP-Glucuronosyltransferase Activity Characterization – LC/MS/MS Method

UDP-glucuronosyltransferases (UGTs) are located in the endoplasmic reticulum (microsomes) of liver and other tissues. UGTs contain a transmembrane domain that anchors the protein in the microsomal membrane leaving the enzyme's hydrophobic active site exposed to the microsomal lumen. This lumenal orientation necessitates carrier-mediated transport of UDPGA into the microsomal lumen, *in vivo*. Maximal UGT activity can be obtained *in vitro* by using a detergent (*i.e.*, CHAPS) to disrupt microsomal membranes thus exposing the UGT active site to the substrate and co-factor.

Experimental Conditions	***Typical Conditions***				
	UGT1A1	***UGT1A4***	***UGT1A6***	***UGT1A9***	***UGT2B7***
Source of enzyme	Microsomes				
Incubation volume	0.2 mL				
[CHAPS][1]	0.5 mM				
[Alamethicin]	50 µg/mg protein				
[Tris-HCl, pH 8.0 at RT][1]	100 mM				
[EDTA][1]	1.0 mM				
[$MgCl_2$][1]	10 mM				
[Saccharic acid 1,4-lactone][1]	0.1 mM				
UDPGA[1]	8.0 mM				
Stop Reagent	2% formic acid in acetonitrile				1% perchloric acid
[Protein][1]	50 µg/mL	250 µg/mL	10 µg/mL	50 µg/mL	50 µg/mL
Substrate	β-Estradiol	Trifluoperazine	Naphthol	Propofol	Morphine
[Substrate][1]	0.1 mM	0.025 mM	0.5 mM	0.05 mM	1.0 mM
Incubation Time	10 min	5 min	10 min	10 min	10 min
Internal Standard	d_5-estradiol 3-glucuronide	prochlorperazine glucuronide	d_7-naphthyl glucuronide	thymol glucuronide	d_3-morphine 3-glucuronide
[Internal Standard]	1 µM	0.5 µM	0.5 µM	0.5 µM	250 ng/mL
Metabolite	Estradiol 3-glucuronide	Trifluoperazine glucuronide	Naphthyl glucuronide	Propofol glucuronide	Morphine 3-glucuronide

[1]These concentrations refer to the concentration present in the incubation mixtures.

Appendix

Appendix: Subcellular Fractions

UDP-Glucuronosyltransferase Activity Characterization – LC/MS/MS Method (*cont.*)

Incubations are typically conducted in glass culture tubes with a final incubation volume of 200 µL (typically 158 µL buffer solution, 20 µL biological sample, 2 µL substrate solution, and 20 µL UDPGA). Zero-time incubations serve as blanks and zero-time incubations spiked with metabolite standard (typically samples ranging between 0.025 to 5 µM) serve as the metabolite standards. Microsomes (diluted to 20x the final incubation concentration) must be pre-incubated in the presence of an activator. Three basic microsomal activation conditions may be used: native (no activator), CHAPS or alamethicin. Each activator is prepared in 20 mM EDTA, pH 7.4. The microsomal activation is typically performed by adding equal volume of the protein and activator solution (50% microsomes, 50% activator/EDTA) and preincubated for 15-25 minutes, **on ice**, prior to incubation with the substrate.

Liver microsomes are incubated at 37 ± 1°C in 200 µL incubation mixtures containing Tris-HCl (100 mM), EDTA (1.0 mM), $MgCl_2$ (10 mM), D-saccharic acid 1,4-lactone (100 µM), UDPGA (8.0 mM) and substrate at the final concentrations indicated. Reactions are started by the addition of the cofactor, UDPGA, and are usually stopped after zero to 10 minutes by the addition of 175 µL of Stop Reagent. Precipitated protein is removed by centrifugation (400-2500 g_{max} for 5-15 minutes at 5-25°C). A portion of the supernatant fraction is analyzed by LC-MS/MS. Certain experiments may require modifications to these typical incubation conditions (*e.g.*, substrate concentration).

Note: Alamethicin is normally used for protein solubilization (activation) at a concentration of 25 or 50 µg per mg of microsomal protein. The concentration of the alamethicin/EDTA solution should be adjusted to deliver the right amount based on the concentration of protein. For example, in order to perform a UGT assay using 0.1 mg/mL of protein in an incubation volume of 1 mL and with activation by alamethicin at 25 µg/mg protein, the stock protein is first diluted to 2 mg/mL (20x the final incubation concentration, 50 µL volume) with 250 mM sucrose. When equal volume (50 µL) of 50 µg/mL alamethicin/EDTA is added, the protein and alamethicin becomes diluted 1:1, yielding 25 µg alamethicin/mg protein for the activation. At the end of the activation time, other incubation components are then added to make a 1:10 dilution of the activated-microsomes.

Aqueous alamethicin solution at 250 µg/mL will precipitate. Hence, where high concentrations of protein are used, a stock solution of 50 mg/mL alamethicin in methanol may be prepared, which can be added directly to the protein, provided the final concentration of organic solvent is $<1\%$ (v/v).

Glucuronidation of 4-Methylumbelliferone – LC/MS/MS Method

The glucuronidation of 4-methylumbelliferone has been shown to be catalyzed by UGT1A6/7 in rat, UGT1A1/6/7 in mouse and UGT1A6 and UGT2B8 in human liver.

Incubations are typically conducted in glass culture tubes with a final incubation volume of 200 µL (typically, 158 µL substrate solution, 20 µL biological sample, 2 µL substrate solution, and 20 µL UDPGA). Zero-time incubations serve as blanks and zero-time incubations spiked with 4-methylumbelliferyl β-D-glucuronide serve as metabolite standards. Microsomes (diluted to 20x the final incubation concentration) must be pre-incubated in the presence of CHAPS solubilization buffer[1] (50% microsomes, 50% CHAPS solubilization buffer) for 15-25 minutes, **on ice**, prior to incubation with the substrate.

Liver microsomes (*e.g.*, 0.1 mg/mL) are incubated at 37°C in 200 µL incubation mixtures containing Tris-HCl (100 mM, pH 7.4), EDTA (1.0 mM), $MgCl_2$ (10 mM), D-saccharic acid 1,4-lactone (100 µM), UDPGA (8.0 mM) and 4-methylumbelliferone (1 mM), at the final concentrations indicated. Reactions are started by the addition of the co-factor, UDPGA (8 mM), and are stopped after 10 minutes by the addition of 175 µL of 2% formic acid in acetonitrile with internal standard. Precipitated protein is removed by centrifugation (920 g_{max} for 10 minutes at 10°C). A portion of the supernatant fraction is analyzed by LC/MS/MS.

[1] *0.5 mM CHAPS in 20 mM EDTA*

Monoamine Oxidase Activity Characterization – LC/MS/MS Method

5-Hydroxytryptamine Oxidation (Monoamine Oxidase-A activity)

Liver mitochondria (*e.g.*, 1.0 mg/mL mg) are incubated at 37 ± 1°C in a 200 µL incubation mixture (final volume) containing potassium phosphate buffer, pH 7.4 (10 mM) and 5-hydroxytryptamine (typically 500 µM). The reactions are started by the addition of mitochondria (typically 20 µL) to the incubation tube containing the substrate solution (typically 180 µL). At designated times (*e.g.*, 10 min), reactions are stopped by the addition of 30 mM sodium borohydride in 20% 2-propanol/ acetonitrile solution (typically 100 µL). Samples are then immediately vortexed and allowed to sit at room temperature for approximately 10-15 min. Subsequently, the internal standard (1 µM 5-hydroxytrytophol-d3) is added to each incubation. Zero-time incubations, which contain mitochondrial protein and substrate solution, serve as blanks. If possible, a selected mitochondrial sample is incubated for approximately half and twice the regular incubation period (to verify metabolite formation is proportional to incubation time) and at approximately half and twice the regular protein concentration (to ensure that metabolite formation is proportional to enzyme concentration). Samples are then analyzed by an LC/MS/MS method monitoring the formation of 5-hydroxytrytophol (5HTOL).

Benzylamine Oxidation (Monoamine Oxidase-B activity)

Liver mitochondria (*e.g.*, 1.0 mg/mL mg) are incubated at 37 ± 1°C in a 200 µL incubation mixture (final volume) containing potassium phosphate buffer, 7.4 (10 mM) and 4-(dimethylamino)benzylamine 2HCl (typically 300 µM). The reactions are started by the addition of mitochondria (typically 20 µL) to the incubation tube containing the substrate solution (typically 180 µL). At designated times (*e.g.*, 10 min), reactions are stopped by the addition of Stop Reagent containing Internal Standard[1] (typically 175 µL). Zero-time incubations, which contain mitochondrial protein and substrate solution, serve as blanks. Blanks spiked with metabolite solution (typically 25 µL) serve as the metabolite standard. Volumes of incubation samples are normalized to the volume of standards by the addition of (typically 25 µL) standard blank solution. If possible, a selected mitochondrial sample is incubated for approximately half and twice the regular incubation period (to verify metabolite formation is proportional to incubation time) and at approximately half and twice the regular protein concentration (to ensure that metabolite formation is proportional to enzyme concentration). Samples are then analyzed by an LC/MS/MS method monitoring the formation of 4-(dimethylamino)benzaldehyde.

[1]Stop Reagent plus Internal Standard: 4-(diethylamino)benzaldehyde (50 ng/mL) in acetonitrile containing 2% formic acid

Human Liver Cytosol Characterization Assays

Aldehyde Oxidase Activity Characterization – LC/MS/MS Method

Aldehyde oxidase (AO) is a cytosolic molybdoenzyme that catalyzes the oxidation of aldehydes to carboxylic acids and nitrogen-containing heterocyclic compounds (substituted pyrolles, pteridines, purines, pyridines, pyrimidines) to ketones or aldehydes. During substrate oxidation, the enzyme is reduced and then reoxidized by molecular oxygen derived from water and hence, it functions as a true oxidase. The conversion of phthalazine to 1-phthalazinone (or phthalazone) is used as specific marker reactions for the measurement of aldehyde oxidase activity in cytosolic or S9 fractions.

Appendix: Subcellular Fractions

To measure aldehyde oxidase (AO) activity, liver cytosol samples (0.05 mg/mL) are incubated at 37 ± 1 °C for 1 minute in a 200-µL incubation mixture (final volume) containing potassium phosphate buffer (50 mM, pH 7.4), and phthalazine (25 µM), at the final concentrations indicated. No cofactors are required. The reactions are started by addition of the substrate into the protein or *vice versa*. The reactions are stopped by the addition of 175 µL of stop reagent (2% formic acid in acetonitrile containing internal standard). Zero-time incubations serve as blanks. Blanks spiked with 25 µL of 1-phthalazinone at various concentrations serve as metabolite standards to construct a calibration curve. The sample volumes are normalized to the volume of the calibration standards by adding the appropriate volume of standard blank solution (typically 25 µL). Precipitated protein is removed by centrifugation (920 × *g* for 10 min at 10°C). Samples are then analyzed by an LC/MS/MS method.

N-Acetyltransferase Activity Characterization

N-Acetylation is a major route of biotransformation for xenobiotics containing an aromatic amine (R-NH2) or a hydrazine group (R-NH-NH2). *N*-Acetylation reactions are catalyzed by cytosolic *N*-acetyltransferases, which require the cofactor acetyl-coenzyme A. The reaction occurs in two sequential steps according to a *ping-pong Bi-Bi* mechanism: the acetyl group from acetyl-CoA is first transferred to an active site cysteine residue within an *N*-acetyltransferase with release of coenzyme A; then the acetyl group is transferred from the acetylated enzyme to the amino group of the substrate with regeneration of the enzyme. Human, rabbits and hamsters express only two *N*-acetyltransferases, NAT1 and NAT2. Dog and fox are not able to acetylate xenobiotics. In humans, sulfamethazine is preferentially acetylated by NAT2.

To measure *N*-acetyltransferase 2 (NAT2) activity, liver cytosol samples (0.5 mg/mL) are incubated at 37 ± 1°C for 10 minutes in a 200-µL incubation mixture (final volume) containing high purity water, potassium phosphate buffer, pH 7.4 (50 mM), magnesium chloride (3 mM), EDTA, pH 7.4 (1 mM), dithiothreitol (2 mM), acetyl-CoA regenerating system, and sulfamethazine (600 µM), at the final concentrations indicated. The reactions are started by the addition of acetyl-CoA regenerating system (20 µL), which consists of acetyl-DL-carnitine (4.5 mM), carnitine acetyltransferase (0.1 Units/mL) and acetyl-CoA (0.1 mM). The reactions are stopped by the addition of 175 µL of stop reagent (acetonitrile containing internal standard). Zero-time incubations, which contain cytosolic protein and substrate solution, serve as blanks. Blanks spiked with 25 µL of *N*-acetyl sulfamethazine at various concentrations serve as metabolite standards to construct a calibration curve. Samples are then analyzed by an LC/MS/MS method.

Sulfotransferase Activity Characterization

To measure sulfotransferase activity, liver cytosol samples (0.5 mg/mL) are incubated at 37 ± 1°C for 10 minutes in a 200-µL incubation mixture (final volume) containing high purity water, potassium phosphate buffer, pH 7.4 (50 mM), magnesium chloride (3 mM), EDTA, pH 7.4 (1 mM), adenosine 3′-phosphate 5′-phosphosulfate lithium salt hydrate (PAPS) (10 mM) and 7-hydroxycoumarin (500 µM), at the final concentrations indicated. The reactions are started by the addition of PAPS (20 µL) and stopped after 10 minutes by the addition of 175 µL of stop reagent (acetonitrile containing internal standard). Zero-time incubations, which contain cytosolic protein and substrate solution, serve as blanks. Blanks spiked with 25 µL of 7-hydroxycoumarin sulfate at various concentrations serve as metabolite standards to construct a calibration curve. Samples are then analyzed by an LC/MS/MS method.

Reaction Phenotyping Kit Instructions

The Reaction Phenotyping Kit contains human-derived material. XenoTech accepts only non-transplantable tissue from donors who test negative for HIV 1 and 2, HTLV, and Hepatitis B and C. However, as a precaution, all human-derived samples should be regarded as a potential biohazard and should be stored, handled and discarded accordingly. The Reaction Phenotyping Kit is intended for *in vitro* use only.

Storage

The Reaction Phenotyping Kit should be stored in an ultra-low freezer (-70°C or colder). At ultra-low temperatures, Sekisui XenoTech's human liver microsomes are stable for >12 years.

Freezing and thawing samples

Sekisui XenoTech's human liver microsomes can be frozen and thawed as many as ten times with no apparent loss of P450 activity, as reported by Pearce *et al.*

R Pearce, CJ McIntyre, A Madan, U Sanzgiri, AJ Draper, P Bullock, DC Cook, LA Burton, J Latham, C Nevins and A Parkinson. Effects of freezing, thawing, and storing human liver microsomes on cytochrome P450. *Arch. Biochem. Biophys*, 331, 145-169, 1996

Consequently, after an aliquot of human liver microsomes is taken from a vial, the residual sample can be re-frozen and used at a later date. Care should be taken to keep thawed samples on ice (-4°C), and to return them as quickly as possible to an ultra-low freezer for storage.

Experimental approaches to reaction phenotyping

Four *in vitro* approaches have been developed for reaction phenotyping - correlation analysis, chemical inhibition, antibody inhibition, and metabolism by recombinant human CYPs. Each has its advantages and disadvantages, and a **combination** of approaches is essential to identify the P450 enzyme(s) primarily responsible for metabolizing a drug, new molecular entity, or any other xenobiotic.

Suggested incubation conditions

For drug metabolism studies, liver microsomes can be incubated with a drug under a variety of conditions, therefore, the experimental conditions described below are provided simply as a guide. Reactions are typically carried out in 200µL incubation mixtures that contain the following components at the final concentrations indicated in parentheses:

- Liver microsomes (0.1-0.2mg/mL)
- Substrate (drug or test article under investigation; various concentrations)
- Potassium phosphate buffer (50mM, pH 7.4)
- Magnesium chloride (3.0mM)
- EDTA (1.0mM, pH 7.4)
- Glucose-6-phosphate (5.0mM, pH 7.4)
- Glucose-6-phosphate dehydrogenase (1.0 Unit/mL)
- NADP (1.0mM, pH 7.4)

Liver microsomes are thawed and dispensed at ~4°C. The substrate, phosphate buffer, MgCl2 and EDTA are typically combined and dispensed as a single solution, at ~4°C. The substrate may need to be dissolved in organic solvent. Because organic solvents can inhibit P450 enzymes, the amount of organic solvent should be kept to a minimum (less than 10 µL/mL or 1% of the incubation volume). If the substrate must be added in organic solvent, it should NOT be added to the microsomes directly because high concentrations of solvent can denature cytochrome P450. Substrates dissolved in organic solvents should be either diluted

Reaction Phenotyping Kit Instructions *(cont.)*

with buffer/MgCl2/EDTA solution or added after these components to avoid exposing the microsomes to high concentrations of organic solvent. The last three components (NADPH-generating system), can also be combined and added as a single solution. Alternatively, the three components of the NADPH-generating system can be replaced with NADPH, although this is relatively expensive.

Incubations are typically conducted at 37°C, and are stopped with a denaturant, typically organic solvent or acid. If necessary, precipitated protein is pelleted in a bench-top centrifuge, and the clear supernatant fraction is analyzed (e.g., by LC/MS/MS) for metabolites and/or remaining substrate.

It is desirable to measure the metabolism of a substrate under initial rate conditions. These conditions must be determined experimentally by varying the amount of microsomal protein and incubation time to ascertain whether metabolite formation is directly proportional to time and protein concentration.

Occasionally, there is little or no information on the metabolism of the substrate with which to develop an analytical procedure. In such cases, it may be useful to incubate a fairly high concentration of pooled microsomal protein (e.g., 1mg/mL) with a high concentration of substrate (e.g., 100 µM or higher depending on solubility) for various times (e.g., 0, 5, 10, 15, 30, 45 and 60 minutes) in order to generate sufficient quantities of metabolites for detection purposes. It should be emphasized however, that reaction phenotyping should, if at all possible, be conducted with pharmacologically relevant concentrations of substrate under initial rate conditions. It will be necessary to reevaluate the effects of protein concentration and incubation time on rates of metabolite formation if the concentration of substrate is decreased in subsequent experiments. The sum of all metabolites should constitute less than 20% of the amount of substrate present. Ideally, the amount of substrate consumed during the reaction should be less than 10% in order to measure initial rates of metabolite formation. However, it should be noted that in the case of substrate loss based studies, substrate loss of 20%-40% is targeted.

Once an analytical procedure has been developed and initial rate conditions have been established with the pooled sample of microsomes, the individual samples of microsomes can be examined for their ability to metabolize the compound of interest. Differences in the rates of formation of the drug metabolites are compared with the sample-to-sample variation in CYP, FMO3, and/or UGT activity (based on the information provided with the kit) either by simple regression analysis (r^2 = coefficient of determination) or by Pearson's product moment correlation analysis (r = correlation coefficient), where the marker CYP/FMO/UGT enzyme activity is the independent variable and the rate of formation of drug metabolite is the dependent variable. The latter determination also provides a measure of the statistical significance of any correlations. A high correlation usually identifies the P450 enzyme responsible for generating each metabolite.

Statistically significant correlations should always be confirmed with a visual inspection of the graph because there are two situations that can produce a misleadingly high correlation coefficient: (1) the regression line does not pass through or near the origin, and (2) there is an outlying data point that skews the correlation analysis.

Correlation analysis works particularly well when a single enzyme dominates the formation of a particular metabolite. When two or more CYP enzymes contribute significantly to the metabolism of a drug at pharmacologically relevant concentrations, the identity of the enzymes involved can be assessed by multivariate regression analysis. This approach successfully identifies the enzymes involved when each enzyme contributes 25% or more to metabolite formation, but it will likely not identify an enzyme that contributes only ~10%.

Microsomes

		8 Donors	10 Donors	50 Donors	XTreme 200 (200 Donors)	Genotyped Microsomes (1 Donor)
	Product ID	H0604	H1000 H1500	H0610 H0610-81 H0620 H0630 H0640	H2610 H2610-81 H2620 H2630 H2640	Varies. See **page 28** for specific product IDs.
	Gender	Mixed	Gender-Specific	Mixed	Mixed	Gender-Specific
	Volume/Vial	0.5 mL	0.5 mL	0.5 mL - 50 mL	0.5 mL - 50 mL	0.5 mL
Characterization Provided						
Enzyme	***Marker Substrate Reaction***					
CYP1A2	Phenacetin *O*-dealkylation	✓	✓	✓	✓	✓
CYP2A6	Coumarin 7-hydroxylation	✓	✓	✓	✓	✓
CYP2B6	Bupropion hydroxylation	✓	✓	✓	✓	✓
CYP2C8	Amodiaquine *N*-dealkylation	✓	✓	✓	✓	✓
CYP2C9	Diclofenac 4′-hydroxylation	✓	✓	✓	✓	✓
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	✓	✓	✓	✓	✓
CYP2D6	Dextromethorphan *O*-demethylation	✓	✓	✓	✓	✓
CYP2E1	Chlorzoxazone 6-hydroxylation	✓	✓	✓	✓	✓
CYP3A4/5	Testosterone 6β-hydroxylation	✓	✓	✓	✓	✓
CYP3A4/5	Midazolam 1′-hydroxylation	✓	✓	✓	✓	✓
CYP4A11	Lauric acid 12-hydroxylation	✓	✓	✓	✓	✓
FMO	Benzydamine *N*-Oxidation	✓	✓	✓	✓	
UGT1A1	17β-Estradiol 3-glucuronidation	✓	✓	✓	✓	
UGT1A4	Trifluoperazine glucuronidation	✓	✓	✓	✓	
UGT1A6	1-Naphthol glucuronidation	✓	✓	✓	✓	
UGT1A9	Propofol glucuronidation	✓	✓	✓	✓	
UGT2B7	Morphine 3-glucuronidation	✓	✓	✓	✓	
Reductase	NADPH-cytochrome *c* reductase	✓	✓	✓	✓	✓
K_m/V_{max}/CL_{int} Values						
CYP1A2	Phenacetin				✓	
CYP2A6	Coumarin				✓	
CYP2B6	Bupropion				✓	
CYP2B6	Efavirenz				✓	
CYP2C8	Amodiaquine				✓	
CYP2C9	Diclofenac				✓	
CYP2C19	*S*-Mephenytoin				✓	
CYP2D6	Dextromethorphan				✓	
CYP3A4/5	Testosterone				✓	
CYP3A4/5	Midazolam				✓	
CYP4A11	Lauric acid				✓	
Content						
Cytochrome b_5		✓	✓	✓	✓	✓
CYP450		✓	✓	✓	✓	✓

**All human liver microsomes are supplied at a protein concentration of 20 mg/mL in 250 mM sucrose.*

Appendix

Appendix: Subcellular Fractions

S9 Fractions

		10 Donors	50 Donors	XTreme 200 (200 Donors)
	Product ID	H1000.S9 H1500.S9	H0610.S9 H0620.S9 H0630.S9 H0640.S9	H2610.S9 H2620.S9 H2630.S9 H2640.S9
	Gender	Gender-Specific	Mixed	Mixed
	Volume/Vial	1.0 mL	0.5 mL - 50 mL	0.5 mL - 50 mL
Characterization Provided				
Enzyme	***Marker Substrate Reaction***			
CYP1A2	Phenacetin *O*-dealkylation			✓
CYP2A6	Coumarin 7-hydroxylation			✓
CYP2B6	Bupropion hydroxylation			✓
CYP2C8	Amodiaquine *N*-dealkylation			✓
CYPC29	Diclofenac 4′-hydroxylation			✓
CYP2C19	*S*-Mephenytoin 4′-hydroxylation			✓
CYP2D6	Dextromethorphan *O*-demethylation			✓
CYP2E1	Chlorzoxazone 6-hydroxylation			✓
CYP3A4/5	Testosterone 6β-hydroxylation			✓
CYP3A4/5	Midazolam 1′-hydroxylation			✓
CYP4A11	Lauric acid 12-hydroxylation			✓
CYP	7-Ethoxycoumarin *O*-dealkylation	✓	✓	✓
UGT	4-Methylumbelliferone glucuronidation	✓	✓	✓
GST	1-Chloro-2,4-dinitro-benzene (CDNB)	✓	✓	✓
Aldehyde Oxidase	Phthalazine			✓
Content				
Cytochrome b_5		✓	✓	✓
CYP450		✓	✓	✓

**All human liver S9 fractions are supplied at a protein concentration of 20 mg/mL in 50 mM Tris-HCl, (pH 7.4 at 4°C) containing 150 mM KCl and 2 mM EDTA.*

Cytosol

		10 Donors	50 Donors	XTreme 200 (200 Donors)
	Product ID	H1000.C H1500.C	H0610.C	H2610.C
	Gender	Gender-Specific	Mixed	Mixed
	Volume/Vial	1.0 mL	1.0 mL	1.0 mL
Characterization Provided				
Enzyme	***Marker Substrate Reaction***			
Aldehyde Oxidase	Phthalazine			✓
NAT2	Sulfamethazine *N*-acetylation			✓
SULT	7-Hydroxycoumarin sulfonation			✓

**All human liver cytosol fractions are supplied at a protein concentration of 10 mg/mL in 50 mM Tris-HCl, (pH 7.4 at 4°C) containing 150 mM KCl and 2 mM EDTA.*

Appendix: Animal Subcellular Fractions

Liver

	*Microsomes**	*S9 Fractions***	*Cytosol****
Species Available	Monkey Minipig Dog Rabbit Guinea Pig Rat Hamster Mouse	Monkey Minipig Dog Rabbit Guinea Pig Rat Hamster Mouse	Monkey Minipig Dog Rabbit Guinea Pig Rat Hamster Mouse
Gender	Gender-Specific	Gender-Specific	Gender-Specific
Volume/Vial	0.5 mL	1.0 mL	1.0 mL
Characterization Provided			
Marker Substrate Reaction			
7-Ethoxycoumarin *O*-dealkylation	✓	✓	
4-Methylumbelliferone glucuronidation		✓	
NADPH-cytochrome *c* reductase	✓		
1-Chloro-2, 4-dinitrobenzene-glutathione by glutathione *S*-transferase		✓	
Content			
Cytochrome b_5	✓	✓	
CYP450	✓	✓	

**All animal liver microsomes are supplied at a protein concentration of 20 mg/mL in 250 mM sucrose*
***All animal liver S9 fractions are supplied at a protein concentration of 10 mg/mL in 50 mM Tris-HCl, 150 mM KCl, 2mM EDTA*
****All animal liver cytosol are supplied at a protein concentration of 20 mg/mL in 50 mM Tris-HCl, 150 mM KCl, 2mM EDTA*

Contact us to learn more at **www.xenotech.com** or call us at **913.GET.P450**.

Lysosome and Tritosome Assays

RNase Alert

To detect RNase the RNaseAlert® Lab Test Kit v2 from Life Technologies (Catalog Number: 4479768) was used. Tritosomes were diluted by performing three serial dilutions by diluting 1:10 (sample: Nuclease-free water) each time. For each tritosome sample to be tested 5 µL of 10x RNaseAlert Lab Test Buffer was added to one tube of RNaseAlert Substrate v2 and then 45 µL of test solution was added. Minus-RNase control tubes were made by adding 45 µL Nuclease-free water to tubes of RNaseAlert Substrate v2. Positive control tubes were made by adding 40 µL Nuclease-free water and 5 µL of RNase A to tubes of RNaseAlert Substrate v2. Tubes were vortexed and dispensed into a black walled microtiter plate and incubated for 10 minutes at 37°C. The plate was then loaded into a microtiter plate reader and analyzed at the specified conditions listed in the kit.

Acid Phosphatase

To detect acid phosphatase the Acid Phosphatase Assay Kit from Sigma-Aldrich (Catalog number: CS0740) was used. Tritosomes are diluted using a 20 fold and a 40 fold dilution. The substrate solution was equilibrated to 37°C. The reaction components were added to a 96-well microtiter plate according to the reaction scheme listed in the kit. The plate was then put on a horizontal shaker and incubated for 10 minutes at 37°C. Reactions are stopped by adding 0.2 mL of Stop Solution to the wells, except the wells containing the Standard Solution. The absorption was then measured at 405 nm on a microtiter plate reader.

Cathepsin B

To detect Cathepsin B, enzymatic activity was assayed with a modified protocol using Z-Arg-Arg-7-amido-4-methylcoumarin hydrochloride (Sigma) as substrate (Giusti et al. 2008). Tritosomes are diluted using a 5-fold dilution. The tritosomes were loaded onto a 96-well microtiter plate and mixed with equal volumes of 2x reaction buffer. A range of AMC calibration standards (Enzo) were diluted in 1x reaction buffer and loaded onto the 96-well plate. The plate was then placed on a horizontal shaker and incubated for 10 minutes at 38°C. The substrate was added to each well containing diluted tritosomes and fluorescence (380 nm/460 nm) was measured kinetically for 20 minutes on a microtiter plate reader. Activity was calculated from the linear portion of the fluorescence curve.

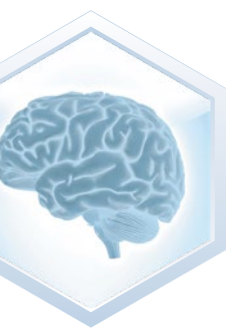
Notes

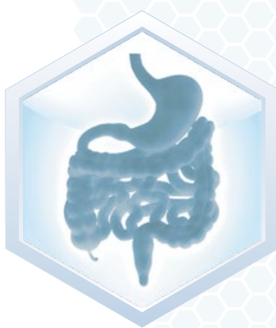
LINES | CYTOSOL | ENZYMES | HEPATOCYTES | HOMOGENATE | KUPFFER CELLS | LYSOSOMES | MAST CELLS | MEDIA | METABOLITES | MICROSOMES

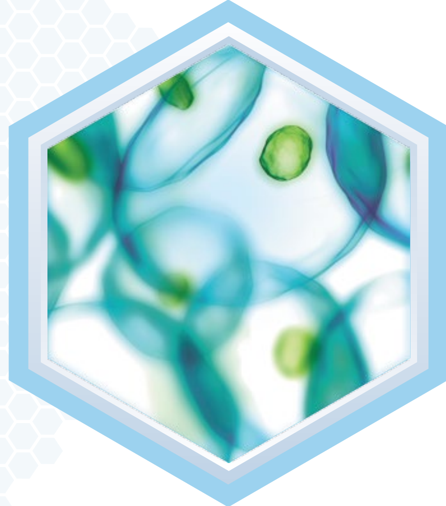
In Vitro Products & Reagents

2017-2018 Technology Guide

SEKISUI XENOTECH

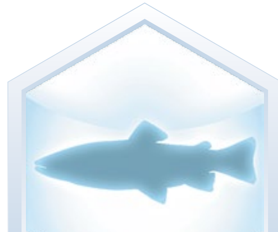






NAL GLAND | BRAIN | COLON | DUODENUM | EYE | HEART | ILEUM | JEJUNUM | KIDNEY | LIVER | LUNG | OVARY | SKIN | SPLEEN | STOMACH | TESTICLE

CANINES | CATS | CHICKENS | COWS | GERBILS | GOATS | GUINEA PIGS | HAMSTERS | HORSES | HUMANS | MICE | MINIPIGS | MONKEYS | PIGS | RABBITS | RATS | SHEEP | TROUT



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About XenoTech

Founded in 1994 by Dr. Andrew Parkinson, XenoTech was spun off as an incubator company from the University of Kansas Medical Center's Pharmacology and Toxicology Department, stemming from Dr. Parkinson's years of research in drug metabolism and cytochromes P450. His focus and dedication to drug metabolism and in-depth understanding of the mechanisms involved in drug-drug interactions formed the high standards of science from which XenoTech has built its reputation.

Everything we do is specialized; our scientific focus, our laboratory facilities, and even the way we organize our staff. Our experience gained from working with hundreds of compounds each year plays a key role in predicting the full victim/perpetrator potential of each compound we investigate. We identify what may happen to a compound when it is metabolized, map out the studies to investigate for any potential safety issues and interpret the results to predict the compound's potential effect in humans.

While pharmaceutical companies are frequent consumers of our products and services, our client list includes a wide range of industry types and sizes. Although the majority of these studies are conducted on drug candidates, XenoTech has also performed studies on pesticides, cosmetics, fragrances, nutraceuticals and herbal preparations.

We are well-known for our broad range of high-quality and often novel *in vitro* products. In addition to our standard product offerings, we prepare and deliver custom-designed products and services in response to your specific research needs.

XenoTech scientists deliver invited seminars, teach drug metabolism courses, conduct hands-on workshops and consult on metabolism-related issues for numerous organizations worldwide, including the FDA. We have extensive experience in the preparation of customized reports for contract service clients from all over the world and can deliver timely reports for electronic or paper submissions. We focus our business, customer service, and support staff on understanding, meeting and exceeding our clients' objectives.

At XenoTech, we pride ourselves on our outstanding record of client retention, and we are committed to continually improving our products and services to meet our clients' changing needs.

Sekisui XenoTech's products and services are managed from our global headquarters in Kansas City, KS, USA. Our new facility boasts 41,500 ft^2, with over 21,000 ft^2 of customized lab space.

Products & Ordering Information

Sekisui XenoTech is one of the largest producers of tissue-derived *in vitro* products in the world. Our experience in preparing *in vitro* test systems dates back to the early 1980s, before XenoTech was even founded. The laboratory that would eventually evolve into XenoTech was busy making microsomes for their own drug metabolism research at the University of Kansas Medical Center. Their research, under the direction of XenoTech founder, Dr. Andrew Parkinson, was so well regarded that after repeated requests from the scientific community, they not only began to perform studies for hire, but also began to prepare and ship microsomes, S9 and hepatocytes to laboratories worldwide.

Today, we continue to prepare these products to meet the high standards we set to support our contract services, which not only serves as a built-in quality control, but gives us an end-user perspective to improve and innovate as we go. We continue to expand our product catalog to include a wide selection of high-quality products to support drug metabolism research, including:

- Subcellular Fractions (Animal and Human Homogenate, Microsomes, S9, Cytosol and Mitochondria)
- Hepatocytes (Animal and Human Cryopreserved, Fresh)
- Kupffer Cells
- Lysosomes / Tritosomes
- Recombinant Enzymes (Cypex)
- Cell Culture Media and Laboratory Reagents
- P450 Substrates and Metabolites (Cypex)

Ordering Information

Online Ordering

The XenoTech eStore was launched in late 2010 as a tool for customers to place product orders easily and effectively using real-time inventory information. The eStore provides full product characterization for easy comparison between lots on all available products. All eStore orders are followed in real-time, showing your order status as it goes through our internal systems until it is shipped.

Online Ordering Features:

- Real-time inventory
- Characterization tables for easy comparison between lots
- Full account access and order history
- Real-time order status updates
- Customer specific pricing

We offer unrivaled quality and selection with an extensive array of products to assist all your *in vitro* ADME research needs. Our standard products feature both subcellular fractions and hepatocytes from many different toxicologically relevant species. We also feature products from Cypex such as recombinant CYP enzymes, P450 substrates and metabolites. Whatever your *in vitro* research needs, Sekisui XenoTech can help.

Sekisui XenoTech is dedicated to providing solutions for your product and research needs. Please direct your inquiries to either our North American or European offices listed below.

Customer Service Department

North America:

1101 W Cambridge Cir Dr
Kansas City, KS 66103
United States
Toll Free: 877.588.7530
Phone: 913.438.7450
Order Fax: 913.227.7171
info@xenotechllc.com

Europe:

Kaplaneigasse 82
64319 Pfungstadt
Germany
Phone: +49 6157 986 3173
Order Fax: +49 6157 986 3174
office@xenotechllc.com

Shipping Policy

Orders will be processed and shipped the next shipping day via FedEx® overnight delivery. If the order is received by 12:00pm CST, same-day shipping is available. Domestic shipments are scheduled Monday-Thursday via FedEx. International shipments will be scheduled to allow proper transit time. Sekisui XenoTech reserves the right to reschedule shipping days for reasons such as inclement weather, national holidays or due to other potential delivery delays.

For a full list of regional account managers and distributors, click on Contact Us at **www.xenotech.com**

**Sekisui XenoTech's eStore is currently for US and Canadian customers only. If you are located outside North America and wish to purchase Sekisui XenoTech products, please contact one of our distributors listed on our website.*

Statement on Informed Consent from Organ Donors

The United Network of Organ Sharing (UNOS) regulates and oversees the use of human tissue intended for transplantation in the United States. Organ donors may elect to have their organs used either for transplantation only, or for transplantation or research. Thus, the donor (or the family of the donor) has the right to prevent the use of the organs for research. Regardless of the use of donated organs, no compensation is given to the donor's family; any such compensation is illegal in the United States. In those cases where donors (or family members) elect to withhold organs from research uses, any organs that cannot be transplanted are discarded.

Seikisui XenoTech receives liver, lung, kidney, intestine, skin and other human tissue from various regional organ procurement organizations that obtain organs approved for research use. Regulations in the United States require that the identity of the donor of organs used for transplantation and research be treated as highly confidential information. Organ procurement organizations maintain the informed consent records from each donor, and our Standard Operating Procedure requires that Sekisui XenoTech personnel confirm the existence of informed consent for research purposes, prior to transport of organs to Sekisui XenoTech. This procedure is intended to ensure that Sekisui XenoTech manufactures products, including human hepatocytes, derived from human organs only when consent has been granted for research use of those specific organs. Following all HIPAA regulations (Health Insurance and Portability and Accountability Act of 1996), Sekisui XenoTech does not, and, in consideration of confidentiality, cannot obtain the informed consent records from these organizations.

All human tissue accepted by XenoTech has tested non-reactive for HIVAb, HBsAg and HCVAb. All human tissue is also tested for CMVAb; due to the ubiquitous nature of CMV exposure, and its relative insignificance as an infectious agent, tissue reactive for CMVAb is accepted. Serology status of each donor is typically determined through the use of ELISA and/or nucleic acid testing by the donating hospital.

Sekisui XenoTech only accepts human tissue from organs approved for research use and verifies all informed consent records obtained by the procurement organizations.

Product Uses

Sekisui XenoTech features one of the most extensive selections of DMPK-focused products on the market. If a study requires tissue-derived products which are not included in Sekisui XenoTech's standard product offering, custom products can be conveniently ordered and are tailored to meet your specific needs.

Applications	*In Vitro* Test Systems								
	Plateable Cryopreserved Hepatocytes	Pooled Cryopreserved Hepatocytes	Individual Cryopreserved Hepatocytes	Kupffer Cells	Pooled S9/Cytosol/ Homogenate	Pooled Microsomes	Individual Microsomes/ S9/Cytosol	Recombinant Enzymes (rCYPS)	Lysosomes/ Tritosomes
Species Differences	+	+++	++	–	++	++	+	–	++
Metabolic Stability	+++	+++	++	–	++	++	+	+	+++
In Vitro Toxicity	++	–	–	+	+++	+	–	–	–
Enzyme Induction	+++	–	–	–	–	–	–	–	–
P450 Inhibition	+	++	+	–	+	+++	+	+	–
Reaction Phenotyping	+	+	+	–	++	+++	+	++	–
Integrated Metabolism	+++	+++	++	–	++	++	+	–	+
Uptake Transporter Assays	+++	+++	++	–	–	–	–	–	–
Efflux Transporter Assays	+++	–	–	–	–	–	–	–	–
Genetic Polymorphisms	+	+++	+	–	–	–	++	+	–
3D Hepatic Models	++	–	++	++	–	–	–	–	–

+++ = *Preferred/best*
++ = *Good*
+ = *Acceptable*
– = *Not recommended*

A full list of individual products and additional details can be found at **www.xenotech.com/products**

Products Supporting *In Vitro* DMPK

Available Products by *In Vitro* Study Type

In Vitro Study Type	*Available Sekisui XenoTech Products*
Enzyme Induction	Cryoplateable human/animal hepatocytes, *CryostaX*™ Plateable Pools, fresh human/animal hepatocytes, Ready-Plate™ hepatocytes, treated animal liver microsomes
Hepatic Uptake	*HepatoSure*™ pooled human hepatocytes (n=100) or *CryostaX*™ (n=10/20), *CryostaX*™ Plateable Pools (n=5/10), cryoplateable human hepatocytes, Qualyst Transporter Certified™ human hepatocytes
Hepatic Efflux	Qualyst Transporter Certified™ human hepatocytes, *CryostaX*™ Plateable Pools, cryoplateable human hepatocytes
Enzyme Inhibition	Human liver microsomes: *XTreme 200*-donor pool Human S9, cytosol: *XTreme 200*-donor pool Hepatocytes: *HepatoSure*™, *CryostaX*™, cryoplateable human/animal hepatocytes, Ready-Plate™ hepatocytes Recombinant enzymes: Bactosomes (rCYPs) CYP inhibitors/substrates
Metabolic Stability/Clearance	Human liver microsomes, S9: *XTreme 200* or 50-donor pool Human liver homogenate Hepatocytes: *HepatoSure*™, *CryostaX*™, *CryostaX*™ Plateable Pools, cryoplateable human/animal hepatocytes, Ready-Plate™ hepatocytes Animal microsomes, S9, extrahepatic subcellular fractions Lysosomes/tritosomes
Metabolite Characterization	Human liver microsomes, S9: *XTreme 200* or 50-donor pool, human liver homogenate Hepatocytes: *HepatoSure*™, *CryostaX*™, animal cryopreserved/fresh hepatocytes
Species Comparison	Animal microsomes, S9, hepatocytes
Reaction Phenotyping	Human liver microsomes: *XTreme 200* or 50-donor pool, reaction phenotyping kit, pooled human hepatocytes, Bactosomes (rCYPs)
Reactive Metabolites	Human liver microsomes, S9: *XTreme 200* or 50-donor pool, human liver homogenate Hepatocytes: *HepatoSure*™, *CryostaX*™, human/animal cryopreserved/fresh hepatocytes
Lysosomal Trapping	*HepatoSure*™, cryoplateable human hepatocytes, *CryostaX*™ Plateable Pools Fa2N-4 immortalized hepatocytes
Kupffer Cell Mediated Toxicity/Macrophage Stimulation	Kupffer cells/Kupffer cell thawing and culture media
Polymorphic Enzymes	Geneknown™ human hepatocytes – CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A5, UGT1A1, OATP1B1 Genotyped human liver microsomes – CYP2C9, CYP2C19, CYP2D6, CYP3A5, UGT1A1, UGT1A9
Gender Differences/ Demographic Differences	Male/female specific lots of liver microsomes, across all species Individual donor human liver microsomes and hepatocytes Ethnic and age specific pools available
Enzyme Cofactors	*RapidStart*™ NADPH regenerating system
Hepatocyte Media	Cryopreservation isolation kits, resuspension medium, culture medium and incubation medium
Fresh hepatocytes	Fresh plated human/animal hepatocytes 6, 12, 24, 48 and 96-plate well formats Fresh human/animal suspensions
Custom Products	Human/animal cellular and subcellular products, various tissues/organs. By request only.
Species Comparison	Human, monkey, dog, minipig, rabbit, guinea pig, hamster, rat and mouse

KUPFFER CELLS | LYSOSOMES | MAST CELLS | MEDIA | METABOLITES | MICROSOMES | MITOCHONDRIA | PRE-LYSATE | REAGENTS | S9 FRACTIONS | SUBSTRA

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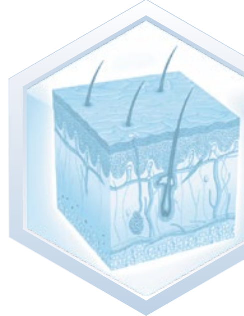

ADRENAL GLAND | BRAIN | COLON | DUODENUM | EYE | HEART | ILEUM | JEJUNUM | KIDNEY | LIVER | LUNG | OVARY | SKIN | SPLEEN | STOMACH | TESTI

CANINES | CATS | CHICKENS | COWS | GERBILS | GOATS | GUINEA PIGS | HAMSTERS | HORSES | HUMANS | MICE | MINIPIGS | MONKEYS | PIGS | RABBITS | RATS | SHEEP | TROUT