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

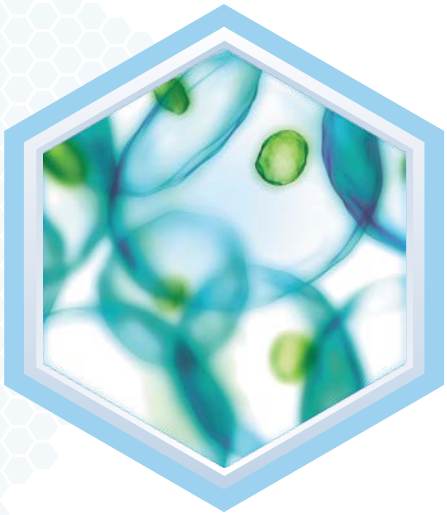
TROUT | SHEEP | RATS | RABBITS | PIGS | MONKEYS | MINIPIGS | MICE | HUMANS | HORSES | HAMSTERS | GUINEA PIGS | GOATS | GERBILS | COWS | CHICKENS | CATS | CANINES

XENOTECH

A BioIVT Company

In Vitro Products & Reagents

2018 Technology Guide



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

XENOTECH
A BioIVT Company

Accelerating the Path to IND with Strategic Science, Quality Products and Global Expertise

Visit us online at www.**xenotech.com**
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About XenoTech

XENOTECH
A BioIVT Company

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.

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

Products & Ordering Information

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 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, XenoTech can help.

XenoTech is dedicated to providing solutions for your product and research needs. Please direct your inquiries to our North American headquarters 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

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. 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**

**XenoTech's eStore is currently for US and Canadian customers only. If you are located outside North America and wish to purchase 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.

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 XenoTech personnel confirm the existence of informed consent for research purposes, prior to transport of organs to XenoTech. This procedure is intended to ensure that 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), XenoTech does not, and, in conside ration 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.

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

Product Uses

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 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 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

Human Hepatocytes

Hepatocytes are widely used in drug discovery and preclinical drug development to perform experiments requiring intact cellular systems. Intact hepatocytes contain the major hepatic drug-metabolizing enzymes required to study the four categories of xenobiotic biotransformation: hydrolysis, reduction, oxidation and conjugation.

XenoTech offers many different hepatocyte products derived from human liver tissue:

- Individual cryoplateable hepatocytes
- CryostaX™ pooled single-freeze hepatocytes for suspension
- CryostaX™ pooled single-freeze plateable hepatocytes
- HepatoSure™ 100-donor pooled hepatocytes
- Geneknown™ cryopreserved hepatocytes
- Transporter characterized hepatocytes
- Individual cryopreserved hepatocytes for suspension
- Fresh hepatocytes
- Ready-Plate™ hepatocytes

For specific characterization information and handling instructions for all human hepatocytes, please reference ***appendix pages 52-60***.

Cryoplateable Hepatocytes

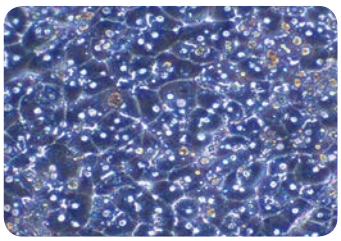
Cryoplateable hepatocytes are the closest alternative to using fresh hepatocytes and are unique for their ability to attach to collagen substratum and respond to prototypical inducers, maintain enzymatic and transporter activities after being cryogenically frozen.

Cryopreservation allows for long-term testing from the same donor while maintaining clinically significant activity levels. Since these cryopreserved hepatocytes are not as time-sensitive as fresh hepatocytes, they can be utilized for long-term metabolic stability studies, enzyme induction and drug transporter studies at your convenience.

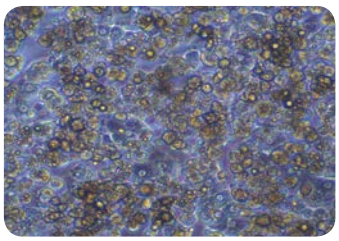
These hepatocytes are characterized for specific CYP activity, general UGT and SULT activities and for induction applications. For specific characterization, see ***appendix page 59***.

Cryoplateable Human Hepatocytes

Product ID	*Description*	*Gender*	*Assured Minimum Yield (AMY)*
H1000.H15B+	Cryoplateable Hepatocytes	Male	4 million
H1000.H15C+	Cryoplateable Hepatocytes	Male	6 million
H1500.H15B+	Cryoplateable Hepatocytes	Female	4 million
H1500.H15C+	Cryoplateable Hepatocytes	Female	6 million



XenoTech cryoplateable lot



Competitor cryoplateable lot

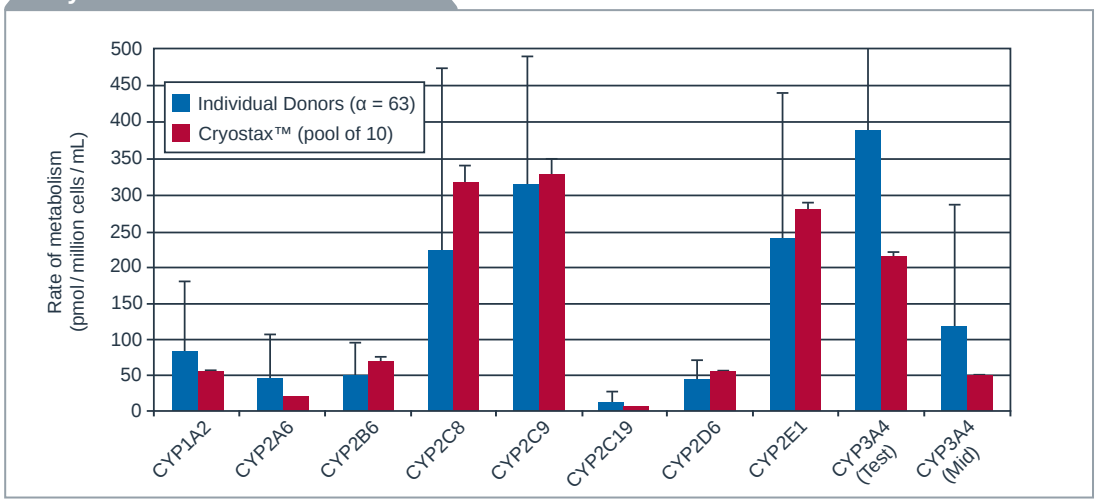
Human Hepatocytes

CryostaX™

Pooled Human Hepatocytes

CryostaX™ is a unique, patented, pooled human hepatocytes product. What makes CryostaX™ unique is that individual donors are represented as pellets. These pellets allow for on-demand custom pooling and large lot sizes, resulting in long-term availability of custom hepatocyte lots. CryostaX™ Pools are ideal for metabolic stability, intrinsic clearance, reaction phenotyping and uptake transporter studies with up to 4 hours of incubation time. Plateable pools are also available.

CryostaX™ vs. Individual Donors



This chart compares the mean of CYP activities from 63 individual human donor lots of cryopreserved hepatocytes versus the CryostaX™ pool of 10. Individual lots were each measured in triplicate. The error bars show the extent of inter-individual variance. CryostaX™ enzymatic activities were each measured in triplicate on three separate days using XenoTech's validated LC/MS/MS methods.

CryostaX™ Plateable Pooled human hepatocytes are recommended for use in extended metabolism studies where long incubation times are required, as well as drug transport studies and induction screening. CryostaX™ Plateable Pools have been extensively characterized for clearance of high, medium and low turnover compounds, as well as uptake transporter activities, and CYP induction. This product is available in pool sizes of 5 and 10 donors, as well as an option to customize your own pool. CryostaX™ Plateable Pools will stay viable in culture for at least 5 days.

For specific CryostaX™ product characterization information, see ***appendix page 59***.

CryostaX™ Human Hepatocytes

Product ID	*Description*	*Pool Size*	*Gender*	*AMY*
HPCH10	CryostaX™ Pooled Hepatocytes, 100 µL pellets	10	Mixed	5 million cells
HPCH20-50	CryostaX™ Pooled Hepatocytes, 50 µL pellets	20	Mixed	5 million cells
HPCH05+	CryostaX™ Plateable Pools, 100 µL pellets	5	Mixed	5 million cells
HPCH10+	CryostaX™ Plateable Pools, 100 µL pellets	10	Mixed	5 million cells

HEPATOSURE™
The Sure Shot from Lot to Lot

100-Donor Human Pooled Hepatocytes

XenoTech's HepatoSure™ product is the largest commercially available pool of human hepatocytes, containing 100 donors. The advantages of using a donor pool this large include better enzyme activity averages, long-term availability of the same product pool enabling lot-to-lot consistency, and better representation of the average American population. Each individual is significantly represented in the final preparation of this product. HepatoSure™ is ideal for metabolic stability, intrinsic clearance, reaction phenotyping and uptake transporter studies with up to 4 hours of incubation time.

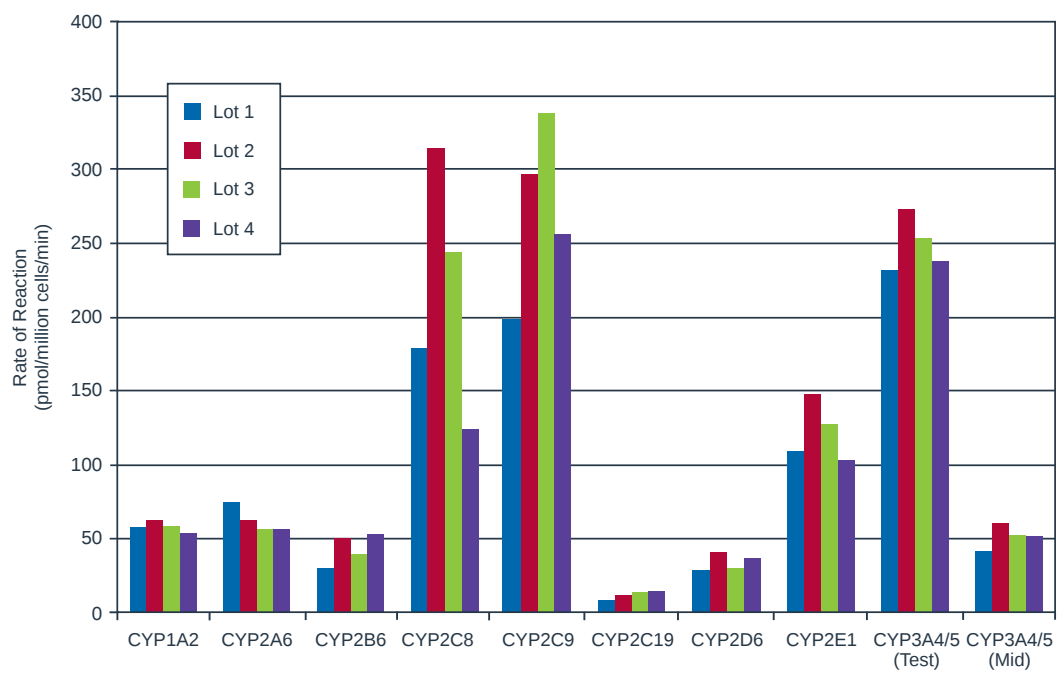
XenoTech characterizes HepatoSure™ for specific CYP and transporter activities, general UGT and SULT activities, intrinsic clearance and aldehyde oxidase activity. For specific characterization, see ***appendix page 59***.

All HepatoSure™ purchases include XenoTech's HepatoSure™ Opti**Thaw** Kit (K8500) at no extra charge to ensure maximum viability and cell yield.

HepatoSure™ Human Hepatocytes

Product ID	*Description*	*Pool Size*	*Gender*	*AMY*
HCP100.H15	HepatoSure™ Pooled Hepatocytes	100	Mixed	5 million cells

Comparison of 4 Pools of HepatoSure™



This figure is a comparison of major P450 activities across 4 different pools of HepatoSure™. Due to the large donor size of the pool, XenoTech is able to minimize activity variability from one lot to the next.

Human Hepatocytes

Geneknown™ Genetically-Defined Cyropreserved Hepatocytes

XenoTech's Geneknown™ hepatocytes are characterized for pharmacologically relevant polymorphisms affecting the ADMET properties of xenobiotics.

Geneknown™ cyropreserved human hepatocytes are offered as pools of cryopreserved cells from multiple donors assembled to study the effects of individual genetic variants. Geneknown™ hepatocytes are cryopreserved using the same proprietary single-freeze method that we use with our CryostaX® product.

To view specific characterization information for our Geneknown™ hepatocytes, see ***appendix page 59.***

The following genes were evaluated for the presence of significant alleles and SNPs (single nucleotide polymorphisms):

- **CYP1A1, 1A2, 2A6, 2B6, 2D6, 2C8, 2C9, 2C19, 2E1, 3A4, 3A5**
- **MDR1** (ABCB1), **MRP2** (ABCC2), **BCRP** (ABCG2), **PEPT2** (SLC15A2), **OCT1** (SLC22A1), **OCT2** (SLC22A2), **OAT1** (SLC22A6), **OATP1B1** (SLCO1B1), **OATP1B3** (SLCO1B3), **OATP2B1** (SLCO2B1)
- **DPYD** (dihydropyrimidine dehydrogenase), **GSTP1** (glutathione S-transferase pi 1), **NAT1** (N-acetyltransferase 1), **NAT2** (N-acetyltransferase 2), **TPMT** (thiopurine S-methyltransferase), **UGT1A1** (UDP glucuonosyltransferase 1 family polypeptide A1), **UGT2B15** (UDP glucuronosyl-transferase 2 family polypeptide B15), **UGT2B7** (UDP glucuronosyltransferase 2 family polypeptide B7), **VKORC1** (vitamin K epoxide reductase complex subunit 1)

Geneknown™ Human Hepatocytes

Product ID	*Description*	*AMY*
HPCH.2C8.HA	Geneknown™ CYP2C8 Hepatocytes - High Activity (*1/*1)	4.5 million cells
HPCH.2C8.MA	Geneknown™ CYP2C8 Hepatocytes - Medium Activity (*3/*3, *3/*4, *4/*4)	4.5 million cells
HPCH.2C9.HA	Geneknown™ CYP2C9 Hepatocytes - High Activity (*1/*1)	4.5 million cells
HPCH.2C9.MA	Geneknown™ CYP2C9 Hepatocytes - Moderate Activity	4.5 million cells
HPCH.2C19.HA	Geneknown™ CYP2C19 Hepatocytes - High Activity (*1/*1)	4.5 million cells
HPCH.2C19.MA	Geneknown™ CYP2C19 Hepatocytes - Moderate Activity (*1/*2)	4.5 million cells
HPCH.2C19.NA	Geneknown™ CYP2C19 Hepatocytes - No Activity (*2/*2)	4.5 million cells
HPCH.2D6.HA	Geneknown™ CYP2D6 Hepatocytes - High Activity (Activity Score > 2.5)	4.5 million cells
HPCH.2D6.MA	Geneknown™ CYP2D6 Hepatocytes - Moderate Activity (Activity Score = 0 - 2.5)	4.5 million cells
HPCH.2D6.NA	Geneknown™ CYP2D6 Hepatocytes - No Activity (Activity Score = 0)	4.5 million cells
HPCH.3A5.HA	Geneknown™ CYP3A5 Hepatocytes - High Activity (*1/*1)	4.5 million cells
HPCH.3A5.MA	Geneknown™ CYP3A5 Hepatocytes - Moderate Activity (*1/*3)	4.5 million cells
HPCH.3A5.NA	Geneknown™ CYP3A5 Hepatocytes - No Activity (*3/*3)	4.5 million cells
HPCH.UGT1A1.HA	Geneknown™ UGT1A1 Hepatocytes - High Activity (*1/*1)	4.5 million cells
HPCH.UGT1A1.MA	Geneknown™ UGT1A1 Hepatocytes - Moderate Activity (*1/*28)	4.5 million cells
HPCH.OATP1B1.HA	Geneknown™ OATP1B1 Hepatocytes - High Activity	4.5 million cells
HPCH.OATP1B1.MA	Geneknown™ OATP1B1 Hepatocytes - Moderate Activity	4.5 million cells

Single Donor Cryopreserved for Suspension Hepatocytes

XenoTech features individual hepatocyte products which allow customers to select donors based on enzyme activity, demographics, age, gender and many other unique characteristics. These products are ideal for metabolic stability, intrinsic clearance, reaction phenotyping and uptake transporter studies with up to 4 hours of incubation time.

XenoTech characterizes for specific CYP activity and general UGT and SULT activities. For specific characterization, see ***appendix page 59.***

Cryopreserved Hepatocytes for Suspension

Product ID	*Description*	*Gender*	*AMY*
H1000.H15-3	Individual Cryopreserved Hepatocytes for Suspension	Male	3 million
H1000.H15B	Individual Cryopreserved Hepatocytes for Suspension	Male	4 million
H1000.H15C	Individual Cryopreserved Hepatocytes for Suspension	Male	6 million
H1500.H15-3	Individual Cryopreserved Hepatocytes for Suspension	Female	3 million
H1500.H15B	Individual Cryopreserved Hepatocytes for Suspension	Female	4 million
H1500.H15C	Individual Cryopreserved Hepatocytes for Suspension	Female	6 million

Immortalized Hepatocytes

XenoTech also offers Fa2N-4 immortalized human hepatocytes, which retain near-normal morphology and function in culture, and allow you to compare results using hepatocytes from the same donor over multiple years and across studies, providing consistent results and the convenience of a predictable supply.

These Fa2N-4 immortalized human hepatocytes are useful to evaluate the induction of major cytochrome P450s (CYPs), UGTs and P-gps, and are a suitable test system for hepatotoxicity assessment, and lysosomal sequestration / trapping.

Immortalized Hepatocytes

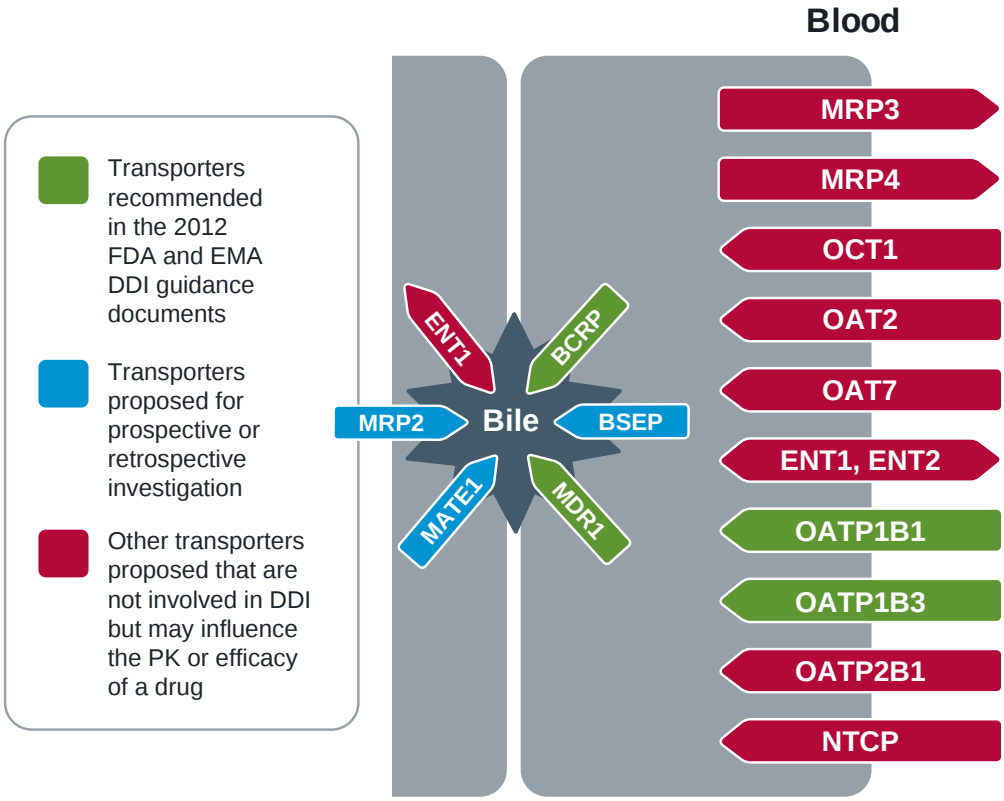
Product ID	*Description*
IFH15	Cryopreserved Fa2N-4 Line, Immortalized Hepatocytes
IFHP06	Fa2N-4 Line, 6-Well, Collagen Coated Plate
IFHP12	Fa2N-4 Line, 12-Well, Collagen Coated Plate
IFHP24	Fa2N-4 Line, 24-Well, Collagen Coated Plate
IFHP48	Fa2N-4 Line, 48-Well Collagen Coated Plate
IFHP96	Fa2N-4 Line, 96-Well, Collagen Coated Plate

Human Hepatocytes

Uptake Transporter Characterized Hepatocytes

The uptake of a drug into and efflux out of hepatocytes can determine the rate at which the molecule is metabolized and eliminated from the body. The uptake transporters, including organic anion transporting polypeptides OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8) OATP2B1 (OATP-B), Na+-taurocholate co-transporting polypeptide (NTCP), organic cation transporter OCT1 and organic anion transporter OAT2, are expressed on the sinusoidal membrane of hepatocytes. These molecules are solute-linked carriers which use an electrochemical gradient of one solute to pump another molecule across the cell membranes and against its concentration gradient.

Uptake Transporter Diagram



Human hepatocytes in suspension can be used to evaluate active uptake of compounds into the cells. These cells are characterized for OATP1B1, OATP1B3, OCT1 and NTCP activity. For full characterization information provided with uptake transporter hepatocytes, please see ***appendix page 59***.

Uptake Transporter Characterized Hepatocytes

Product ID	*Description*	*Gender*	*AMY*
H1000.H15T	Uptake Transporter-Characterized Human Hepatocytes	Male	4-6 million cells
H1500.H15T	Uptake Transporter-Characterized Human Hepatocytes	Female	4-6 million cells

Qualyst Transporter Certified™ Cryoplateable Hepatocytes

Select lots of XenoTech cryoplateable human hepatocytes have been certified to culture for 5 days and maintain both uptake and efflux transporters as well as proper bile canaliculi formation. The Transporter Certified™ hepatocytes have passed a multi-point evaluation starting with plating/culturing characteristics and ending with a broad evaluation of hepatic functions including uptake and efflux transporter functions, metabolic function and baseline bile acid synthesis/production. Aside from just using high quality cells in your study, these cells can also be used in B-CLEAR® studies with a Qualyst Transporter Solutions Cell-Less B-CLEAR® Kit.

Which Functions are Evaluated During Transporter Certification?

- Hepatic Transport
  - OATPs (uptake)
  - NTCP (uptake)
  - OCT1 (uptake)
  - BSEP (efflux)
  - P-gp (efflux)
  - MRP2 (efflux)
  - BCRP (efflux)
  - MATE1 (efflux)
- Glucuronidation
- Bile Acid Synthesis/Production

Cell-less B-CLEAR® kits can be purchased from Qualyst Transporter Solutions and contain the following:

- Cryopreserved hepatocyte thawing medium
- Hepatocyte seeding medium
- Hepatocyte overlay medium
- Hepatocyte maintenance medium
- Assay buffers

Qualyst Transporter Certified™ Cryoplateable Hepatocytes

Product ID	*Description*	*Gender*	*AMY*
H1000.H15Q	Qualyst Transporter Certified™ Cryoplateable Human Hepatocytes	Male	4-6 million
H1500.H15Q	Qualyst Transporter Certified™ Cryoplateable Human Hepatocytes	Female	4-6 million

Human Hepatocytes

Ready-Plate™ Human Hepatocytes

XenoTech's Ready-Plate™ human hepatocytes provide a convenient alternative to fresh hepatocytes. These pre-characterized, cryoplateable human hepatocytes are delivered to you in the well-format of your choice, on demand. Choose from our large selection of characterized cryoplateable hepatocytes and let our cell culture experts take care of the rest!

XenoTech experts will thaw, plate, overlay with extracellular matrix and deliver to your door; saving you valuable time and ensuring a perfect plate every time.

All of our Ready-Plate™ hepatocytes must demonstrate great morphology, confluency and meet our stringent acceptance criteria for enzymatic activity and fold induction in response to prototypical inducers. Each culture is evaluated by XenoTech's Quality Control department prior to shipment to ensure only the best quality hepatocytes are shipped to our customers. Upon arrival, these cultures are ready to use for induction assays, uptake and efflux assays as well as long-term metabolism assays.

We can plate a single donor, several donors or pre-pool and plate donors to create a true custom-tailored plate. All orders will be processed according to the following schedule*:

Monday: Ready-Plate™ hepatocytes prepared and plated
Tuesday: Ready-Plate™ hepatocytes shipped
Wednesday: Ready-Plate™ hepatocytes delivered by 10:00 AM

*Alternative shipping options/schedules are available. **Contact us for custom shipping schedules.**

Ready-Plate™ Hepatocytes

Product ID	*Description*	*Format*
CHP06	Ready-Plate™ Human Hepatocytes with Matrigel Overlay	6-well
CHP12	Ready-Plate™ Human Hepatocytes with Matrigel Overlay	12-well
CHP24	Ready-Plate™ Human Hepatocytes with Matrigel Overlay	24-well
CHP48	Ready-Plate™ Human Hepatocytes with Matrigel Overlay	48-well
CHP96	Ready-Plate™ Human Hepatocytes with Matrigel Overlay	96-well

Fresh Plated Hepatocytes

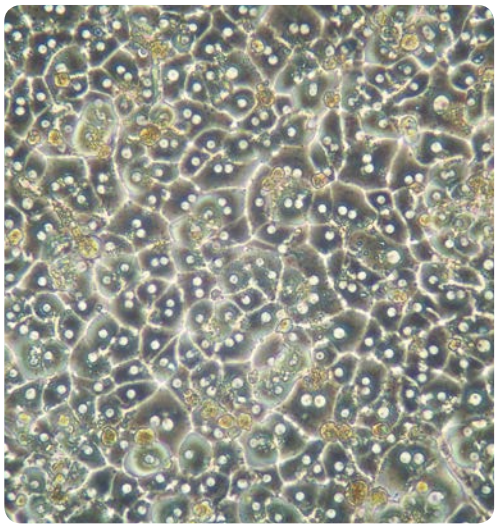
Fresh plated hepatocytes respond to prototypical enzyme inducers in a predictable manner and are useful for evaluating the enzyme induction potential of drug candidates as well as conducting clearance and toxicity studies. They can also be cultured over extended periods of time while maintaining hepatic specific functions for longer studies. XenoTech is able to produce custom plating formats to fit your research needs.

Fresh plated hepatocytes are characterized for CYP induction activity after treatment with prototypical inducers. For more information on specific characterization, see ***appendix page 60***.

Fresh Plated Human Hepatocytes

Product ID	*Description*	*Format*
HHP06M	Fresh Hepatocyte 6-well Collagen-coated Plate with Matrigel Overlay	6-well
HHP12M	Fresh Hepatocyte 12-well Collagen-coated Plate with Matrigel Overlay	12-well
HHP24M	Fresh Hepatocyte 24-well Collagen-coated Plate with Matrigel Overlay	24-well
HHP48M	Fresh Hepatocyte 48-well Collagen-coated Plate with Matrigel Overlay	48-well
HHP96M	Fresh Hepatocyte 96-well Collagen-coated Plate with Matrigel Overlay	96-well
HHS01	Fresh Human Hepatocyte Suspensions, 10 Million Cell Minimum	Vial

**Hepatocytes available without Matrigel overlay upon request.*



Representative lot of fresh human hepatocytes

Due to the time-sensitive nature of fresh hepatocytes, XenoTech customers may sign up for **Liver Alert** emails. These email notifications are sent out when fresh human hepatocytes become available. Visit **xenotech.com/liver-alert** to sign up or contact our customer service office at **913.GET.P450**.

Animal Hepatocytes

XenoTech offers a variety of hepatocytes from clinically-relevant species and features cryopreserved and fresh animal products. XenoTech also prepares custom animal hepatocytes for customers requiring samples that aren't included in our standard product line.

For specific animal hepatocyte characterization information, please reference ***appendix page 61***. Handling instructions for all hepatocytes can be found on ***appendix pages 52-58***.

Cryoplateable Hepatocytes

XenoTech offers cryoplateable animal hepatocytes from clinically-relevant species including monkey, rat and mouse.

These hepatocytes are characterized for general CYP, UGT and SULT activities. For specific cryoplateable animal hepatocyte characterization information, see ***appendix page 61***.

Cryoplateable Monkey – Cynomolgus

Product ID	*Description*	*Gender*	*AMY*
P2000.H15A+	Cryoplateable Cynomolgus Monkey Hepatocytes	Male	4 million
P2000.H15B+	Cryoplateable Cynomolgus Monkey Hepatocytes	Male	6 million

Cryoplateable Rat – IGS Sprague-Dawley / Wistar / Wistar Han

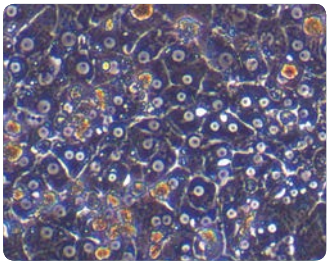
Product ID	*Description*	*Gender*	*AMY*
R1000.H15+	Cryoplateable IGS Sprague-Dawley Rat Hepatocytes	Male	7 million
R3000.H15+	Cryoplateable Wistar Rat Hepatocytes	Male	7 million
R6000.H15+	Cryoplateable Wistar Han Rat Hepatocytes	Male	7 million

Cryoplateable Mouse – CD-1

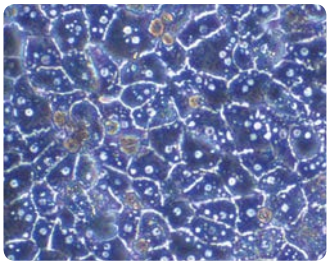
Product ID	*Description*	*Gender*	*AMY*
M1000.H15+	Cryoplateable CD-1 Mouse Hepatocytes	Male	2 million
M1000.H15B+	Cryoplateable CD-1 Mouse Hepatocytes	Male	4 million
M1500.H15+	Cryoplateable CD-1 Mouse Hepatocytes	Female	2 million

Cryoplateable Dog – Beagle

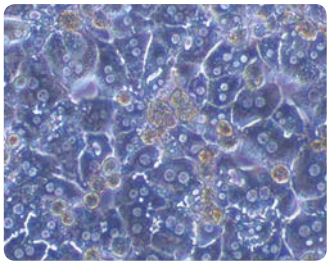
Product ID	*Description*	*Gender*	*AMY*
D1000.H15B+	Cryoplateable Beagle Dog Hepatocytes	Male	6 million



Monkey cryoplateable lot



Rat cryoplateable lot



Mouse cryoplateable lot

Cryopreserved Pooled Hepatocytes for Suspension

XenoTech offers cryopreserved animal hepatocytes from clinically-relevant species including monkey, minipig, dog, rabbit, rat and mouse.

These hepatocytes are characterized for general CYP, UGT and SULT activities. For specific characterization information, see ***appendix page 61.***

Cryopreserved Monkey – Rhesus / Cynomolgus

Product ID	*Description*	*Gender*	*AMY*
P1000.H15A	Cryopreserved Rhesus Monkey Hepatocytes	Male	4 million
P1000.H15B	Cryopreserved Rhesus Monkey Hepatocytes	Male	6 million
P1500.H15A	Cryopreserved Rhesus Monkey Hepatocytes	Female	4 million
P1500.H15B	Cryopreserved Rhesus Monkey Hepatocytes	Female	6 million
P2000.H15A	Cryopreserved Cynomolgus Monkey Hepatocytes	Male	4 million
P2000.H15B	Cryopreserved Cynomolgus Monkey Hepatocytes	Male	6 million
P2500.H15A	Cryopreserved Cynomolgus Monkey Hepatocytes	Female	4 million
P2500.H15B	Cryopreserved Cynomolgus Monkey Hepatocytes	Female	6 million

Cryopreserved Minipig – Gottingen

Product ID	*Description*	*Gender*	*AMY*
Z6000.H15	Cryopreserved Gottingen Minipig Hepatocytes	Male	4 million

Cryopreserved Dog – Beagle

Product ID	*Description*	*Gender*	*AMY*
D1000.H15	Cryopreserved Beagle Dog Hepatocytes	Male	3.5 million
D1000.H15B	Cryopreserved Beagle Dog Hepatocytes	Male	6 million
D1500.H15	Cryopreserved Beagle Dog Hepatocytes	Female	3.5 million

Cryopreserved Rabbit – New Zealand White

Product ID	*Description*	*Gender*	*AMY*
L1000.H15	Cryopreserved New Zealand White Rabbit Hepatocytes	Male	5 million
L1500.H15	Cryopreserved New Zealand White Rabbit Hepatocytes	Female	5 million

Cryopreserved Rat – IGS Sprague-Dawley / Wistar / Wistar Han

Product ID	*Description*	*Gender*	*AMY*
R1000.H15	Cryopreserved IGS Sprague-Dawley Rat Hepatocytes	Male	7 million
R1500.H15	Cryopreserved IGS Sprague-Dawley Rat Hepatocytes	Female	7 million
R3000.H15	Cryopreserved Wistar Rat Hepatocytes	Male	7 million
R6000.H15	Cryopreserved Wistar Han Rat Hepatocytes	Male	7 million

Cryopreserved Mouse – CD-1

Product ID	*Description*	*Gender*	*AMY*
M1000.H15	Cryopreserved CD-1 Mouse Hepatocytes	Male	2 million
M1000.H15B	Cryopreserved CD-1 Mouse Hepatocytes	Male	4 million
M1500.H15	Cryopreserved CD-1 Mouse Hepatocytes	Female	2 million

Animal Hepatocytes

Ready-Plate™ Animal Hepatocytes

XenoTech's Ready-Plate™ animal hepatocytes provide a convenient alternative to fresh hepatocytes. These pre-characterized, cryoplateable animal hepatocytes are delivered to you in the well-format of your choice, on demand. Choose from our large selection of characterized cryoplateable hepatocytes and let our cell culture experts take care of the rest!

XenoTech experts will thaw, plate, overlay with extracellular matrix and deliver to your door; saving you valuable time and ensuring a perfect plate every time.

All of our Ready-Plate™ hepatocytes must demonstrate great morphology, confluency and meet our stringent enzymatic activity criteria. We can plate a single species or multiple species to create a custom-tailored plate. Orders will be processed according to the following schedule unless otherwise requested: Prepared and plated **Monday**, shipped **Tuesday**, delivered by 10:00 AM **Wednesday**.

Ready-Plate™ Monkey – Cynomolgus

Product ID	*Description*	*Format*
CPP06	Ready-Plate™ Cynomolgus Monkey Hepatocytes with Matrigel Overlay	6-well
CPP12	Ready-Plate™ Cynomolgus Monkey Hepatocytes with Matrigel Overlay	12-well
CPP24	Ready-Plate™ Cynomolgus Monkey Hepatocytes with Matrigel Overlay	24-well
CPP48	Ready-Plate™ Cynomolgus Monkey Hepatocytes with Matrigel Overlay	48-well
CPP96	Ready-Plate™ Cynomolgus Monkey Hepatocytes with Matrigel Overlay	96-well

Ready-Plate™ Rat – IGS Sprague-Dawley

Product ID	*Description*	*Format*
CRP06	Ready-Plate™ IGS Sprague-Dawley Rat Hepatocytes with Matrigel Overlay	6-well
CRP12	Ready-Plate™ IGS Sprague-Dawley Rat Hepatocytes with Matrigel Overlay	12-well
CRP24	Ready-Plate™ IGS Sprague-Dawley Rat Hepatocytes with Matrigel Overlay	24-well
CRP48	Ready-Plate™ IGS Sprague-Dawley Rat Hepatocytes with Matrigel Overlay	48-well
CRP96	Ready-Plate™ IGS Sprague-Dawley Rat Hepatocytes with Matrigel Overlay	96-well

Ready-Plate™ Mouse – CD-1

Product ID	*Description*	*Format*
CMP06	Ready-Plate™ CD-1 Mouse Hepatocytes with Matrigel Overlay	6-well
CMP12	Ready-Plate™ CD-1 Mouse Hepatocytes with Matrigel Overlay	12-well
CMP24	Ready-Plate™ CD-1 Mouse Hepatocytes with Matrigel Overlay	24-well
CMP48	Ready-Plate™ CD-1 Mouse Hepatocytes with Matrigel Overlay	48-well
CMP96	Ready-Plate™ CD-1 Mouse Hepatocytes with Matrigel Overlay	96-well

Ready-Plate™ Dog – Beagle

Product ID	*Description*	*Format*
CDP06	Ready-Plate™ Beagle Dog Hepatocytes with Matrigel Overlay	6-well
CDP12	Ready-Plate™ Beagle Dog Hepatocytes with Matrigel Overlay	12-well
CDP24	Ready-Plate™ Beagle Dog Hepatocytes with Matrigel Overlay	24-well
CDP48	Ready-Plate™ Beagle Dog Hepatocytes with Matrigel Overlay	48-well
CDP96	Ready-Plate™ Beagle Dog Hepatocytes with Matrigel Overlay	96-well

Fresh Animal Hepatocytes

Fresh plated hepatocytes respond to prototypical enzyme inducers in a predictable manner and are useful for studying the enzyme-inducing potential of drug candidates. They are also well-suited for conducting clearance and toxicity studies.

For specific characterization information, please reference ***appendix page 61***.

Fresh Rat – IGS Sprague-Dawley

Product ID	*Description*	*Format*
RHP06M	Fresh IGS Sprague-Dawley Rat Hepatocytes, Collagen-coated Plates with Matrigel Overlay	6-Well
RHP12M	Fresh IGS Sprague-Dawley Rat Hepatocytes, Collagen-coated Plates with Matrigel Overlay	12-Well
RHP24M	Fresh IGS Sprague-Dawley Rat Hepatocytes, Collagen-coated Plates with Matrigel Overlay	24-Well
RHP48M	Fresh IGS Sprague-Dawley Rat Hepatocytes, Collagen-coated Plates with Matrigel Overlay	48-Well
RHP96M	Fresh IGS Sprague-Dawley Rat Hepatocytes, Collagen-coated Plates with Matrigel Overlay	96-Well

**Hepatocytes available without Matrigel overlay upon request.*

Fresh Mouse – CD-1

Product ID	*Description*	*Format*
MHP06M	Fresh CD-1 Mouse Hepatocytes, Collagen-coated Plates with Matrigel Overlay	6-Well
MHP12M	Fresh CD-1 Mouse Hepatocytes, Collagen-coated Plates with Matrigel Overlay	12-Well
MHP24M	Fresh CD-1 Mouse Hepatocytes, Collagen-coated Plates with Matrigel Overlay	24-Well
MHP48M	Fresh CD-1 Mouse Hepatocytes, Collagen-coated Plates with Matrigel Overlay	48-Well
MHP96M	Fresh CD-1 Mouse Hepatocytes, Collagen-coated Plates with Matrigel Overlay	96-Well

**Hepatocytes available without Matrigel overlay upon request.*

Cell Media

XenoTech offers special media for thawing and isolating our cryopreserved hepatocytes and for various applications or procedures. These media include isolation kits, thawing kits, re-suspension media, culture media and incubation media and are optimized for use with XenoTech hepatocytes.

Cell Media

Product ID	*Product Name*	*Volume*	*Product Details*
K8000	Opti**Thaw** Hepatocyte Kit	47 mL	Proprietary cell culture media optimized for thawing cryopreserved hepatocytes, plus Trypan blue solution to obtain accurate cell yield and viability.
K8100	Opti**Thaw** Mouse Hepatocyte Kit	45 mL	Proprietary culture media optimized for thawing cryopreserved mouse hepatocytes, plus Trypan blue solution to obtain accurate cell yield and viability.
K8200	Opti**Plate** Hepatocyte Media	40 mL	Proprietary culture media optimized for plating hepatocytes.
K8300	Opti**Culture** Hepatocyte Media	100 mL	Proprietary culture media optimized for culturing plated hepatocytes. This is a serum-free media. The Pen/Strep supplement is packaged separately for addition when ready to use the media.
K8400	Opti**Incubate** Hepatocyte Media	50 mL	Culture media optimized for incubation of hepatocytes in suspension and plated incubations.
K8500	Hepatosure™ Opti**Thaw** Kit	47 mL	Proprietary cell culture media optimized for thawing Hepatosure™ Pooled cryopreserved hepatocytes, plus Trypan blue solution to obtain accurate cell yield and viability.
K8600 – K8650	Opti**Matrix** Overlay	0.5 – 1.5 mL	Hepatocyte overlay matrix to increase confluency, preserve viability, structure and appearance, assist network formation, and improve excretory function.
K8700	Opti**Thaw** Kupffer Cell Thaw/Culture Media	100 mL	Media for the thawing and culture of Kupffer cells.

Kupffer Cells

Kupffer cells, macrophages endogenous to the liver, have the ability to modulate hepatic inflammation and injury associated with various pathophysiologies and toxicities. Pro-inflammatory cytokines released by activated Kupffer cells, such as Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6), are associated with up-regulation of acute-phase response proteins and suppression of CYP enzymes. For new biological entities, particularly immunomodulators, evaluating the potential for Kupffer cell activation is an emerging concept in preclinical development. Kupffer cells comprise approximately 4-8% of total liver cell content and approximately 20% of non-parenchymal cells.

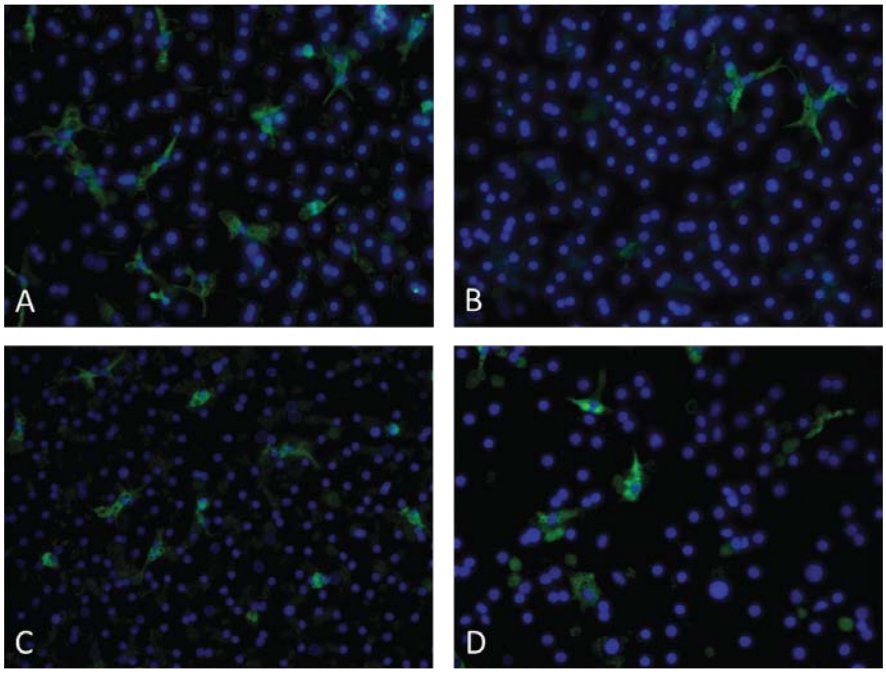
XenoTech offers Kupffer cells to aide in the assessment of cytokine release stimulated by biologics and small molecule drugs. Our Kupffer cells have been characterized for the following:

- **Cell viability and yield** (Trypan blue staining)
- **Functionality:** Activation by bacterial lipopolysaccharide (LPS) measured by induction of TNF-α and IL-6 mRNA and protein levels
- **Purity:** Expression of macrophage marker CD68 (by immuno-fluorescent microscopy)

For more information on our Kupffer cells, please reference appendix ***page 62***.

Kupffer Cells

Product ID	*Description*	*Gender*	*Volume*	*AMY*
HK1000.H15	Cryopreserved Human Male Kupffer Cells	Male	1.0 mL	1 million
HK1500.H15	Cryopreserved Human Female Kupffer Cells	Female	1.0 mL	1 million



(A) 43-year old Caucasian female, 9.3% Kupffer cells; (B) 45-year old Caucasian male, 2.2% Kupffer cells; (C) 7-year old Caucasian male, 3.6% Kupffer cells; (D) 53-year old Hispanic female, 5.4% Kupffer cells. The images shown, selected to emphasize Kupffer cell morphology, are only a part of the field that was used for quantification of the macrophages.

Cell & Tissue Banks

JCRB Cell Bank

XenoTech has teamed up with the National Institute of Biomedical Innovation to bring you access to over 1,100 cell lines, helping you to bridge the gap between research and discovery. XenoTech serves as the exclusive US provider of the Japanese Collection of Research Biosources (JCRB) catalog of cell lines.

JCRB was founded by the Ministry of Health, Labor and Welfare in 1984. They provide one of the most comprehensive cell banks in the world to users across the globe.

Types of cell lines offered include:

- General cells
- Luciferase-expressing cancer cells
- Mouse homozygous mutant ES cells
- Immortalized Mesenchymal stem cells
- Genetically-modified cells

XenoTech is committed to making the ordering process for these cell lines seamless. All aspects of your order will be handled by XenoTech including invoicing and importation. Simply place your order for the desired cell lines and XenoTech takes care of the rest.

JCRB cell lines are imported on a bi-weekly basis. XenoTech will notify you of your estimated product delivery date and invoice you at the time of shipment.

To view the complete list of cell lines offered by XenoTech, visit
www.xenotech.com/products/jcrb-cell-bank

XenoTech Research Biobank

XenoTech's Research Biobank provides pre-lysate tissue and hepatocytes representing normal livers as well as early stages of alcoholic or non-alcoholic fatty liver disease. The specimens are superior to tissue samples collected in a typical post-mortem because they come from organs initially intended for transplantation, are available in quantities larger than those obtained during needle biopsies, and are collected in a timely manner with precise care taken to minimize downtime and preserve tissue viability.

Tissue samples include pathologic diagnosis, demographic, BMI, history of diabetes, alcohol use data and representative microphotographs. H&E slides are prepared for each lot to illustrate tissue conditions and, together with the patient's medical history, to offer a basic diagnosis. Masson's trichrome slides are prepared as justified to establish the presence or absence of liver fibrosis.

Research Biobank Pre-Lysate

Product ID	*Description*	*Amount*
HHPL.NT	Normal Liver Pre-Lysate	0.5g
HHPL.ST	Steatosis Liver Pre-Lysate	0.5g
HHPL.HST	Steatohepatitis Liver Pre-Lysate	0.5g

To view the donor list for the XenoTech Research Biobank, visit
www.xenotech.com/products/research-biobank

Subcellular Fractions

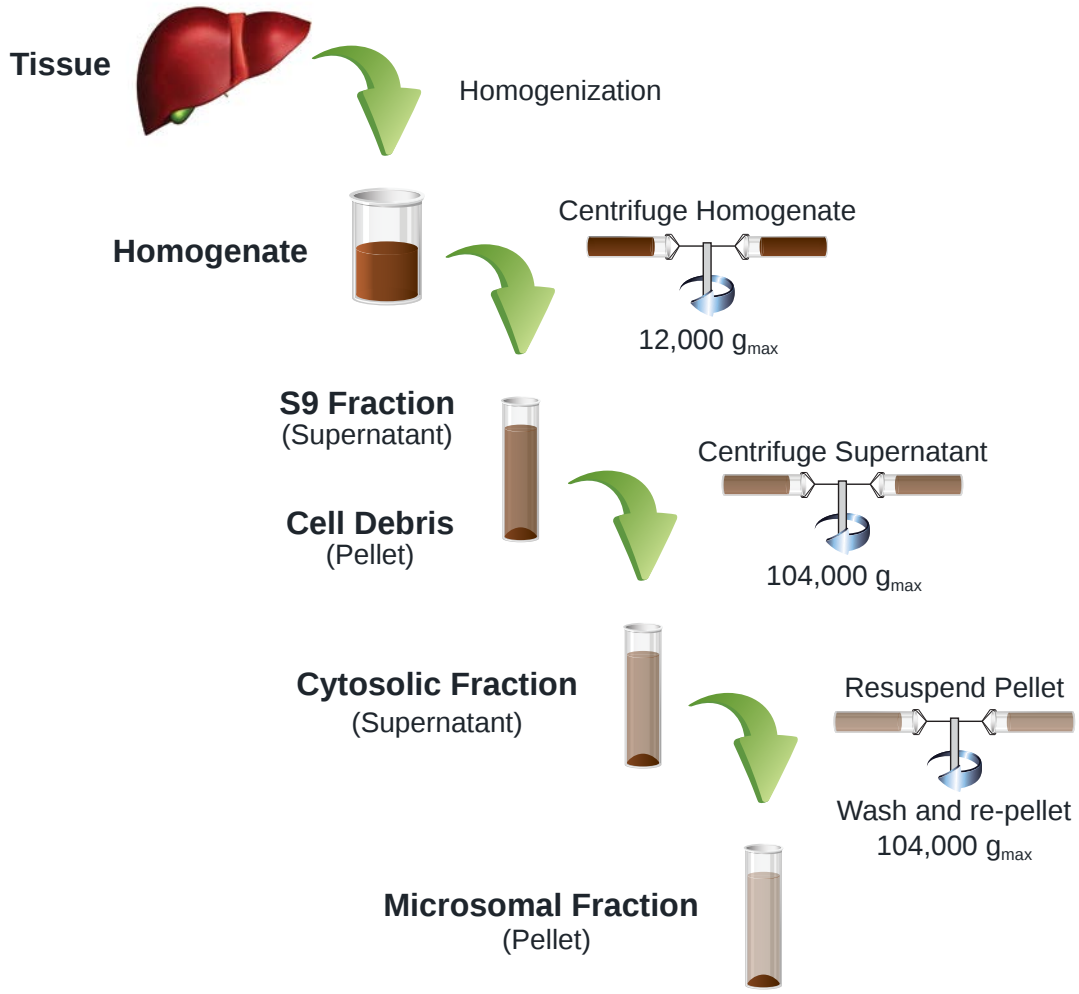
XenoTech's subcellular fractions are widely used in drug discovery and preclinical drug development to evaluate species differences, similarities in metabolite formation by various species, metabolic stability, *in vitro* intrinsic clearance, reaction phenotyping (enzyme mapping) and enzyme inhibition.

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 XenoTech's Quality Control Unit.

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

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

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

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

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

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. 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

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

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

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

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.*

Extrahepatic Subcellular Fractions

Lung Subcellular Fractions

As the primary site for the entrance of airborne agents, and as the recipient of all cardiac output, lungs provide first-pass and/or systemic metabolism for many xenobiotics. The lungs contain several enzymatic pathways including cytochrome P450 and phase II enzymes capable of xenobiotic metabolism.

XenoTech offers subcellular fractions of pulmonary tissue from five toxicologically significant species: human, non-human primate, dog, rat and mouse.

Lung microsomes are supplied in 250 mM sucrose buffer. S9 and cytosolic fractions are supplied in 50 mM Tris-HCl, (pH 7.4 at 4°C), containing 150 mM KCl and 2 mM EDTA.

For more information on our extrahepatic subcellular fractions, including product preparation and characterization data, see ***appendix pages 79-85***.

Lung Human

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
H0610.P(S)	Pooled Human Lung Microsomes, Smokers	Mixed	0.5 mL	10 mg/mL
H0610.PS9(S)	Pooled Human Lung S9, Smokers	Mixed	1.0 mL	5 mg/mL
H0610.PC(S)	Pooled Human Lung Cytosol, Smokers	Mixed	1.0 mL	5 mg/mL
H0610.P(NS)	Pooled Human Lung Microsomes, Non-Smokers	Mixed	0.5 mL	10 mg/mL
H0610.PS9(NS)	Pooled Human Lung S9, Non-Smokers	Mixed	1.0 mL	5 mg/mL
H0610.PC(NS)	Pooled Human Lung Cytosol, Non-Smokers	Mixed	1.0 mL	5 mg/mL

Lung Monkey – Cynomolgus

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
P2000.P	Pooled Cynomolgus Monkey Lung Microsomes	Male	0.5 mL	10 mg/mL
P2000.PS9	Pooled Cynomolgus Monkey Lung S9 Fraction	Male	1.0 mL	5 mg/mL
P2000.PC	Pooled Cynomolgus Monkey Lung Cytosol	Male	1.0 mL	5 mg/mL

Lung Dog – Beagle

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
D1000.P	Pooled Beagle Dog Lung Microsomes	Male	0.5 mL	10 mg/mL
D1000.PS9	Pooled Beagle Dog Lung S9 Fraction	Male	1.0 mL	5 mg/mL
D1000.PC	Pooled Beagle Dog Lung Cytosol	Male	1.0 mL	5 mg/mL

Lung Rat – IGS Sprague-Dawley

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
R1000.P	Pooled IGS Sprague-Dawley Rat Lung Microsomes	Male	0.5 mL	10 mg/mL
R1000.PS9	Pooled IGS Sprague-Dawley Rat Lung S9 Fraction	Male	1.0 mL	5 mg/mL
R1000.PC	Pooled IGS Sprague-Dawley Rat Lung Cytosol	Male	1.0 mL	5 mg/mL

Lung Mouse – CD-1

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
M1000.P	Pooled CD-1 Mouse Lung Microsomes	Male	0.5 mL	10 mg/mL
M1000.PS9	Pooled CD-1 Mouse Lung S9 Fraction	Male	1.0 mL	5 mg/mL
M1000.PC	Pooled CD-1 Mouse Lung Cytosol	Male	1.0 mL	5 mg/mL

Kidney Subcellular Fractions

The kidney has many significant metabolic responsibilities including the hydroxylation of fatty acids and their derivatives. The kidney is also a major site of drug-induced toxicity.

Kidneys express relatively high levels of CYP4A and CYP4B enzymes, the efflux transporter P-glycoprotein (MDR1), and phase II drug-metabolizing enzymes such as UGT1A6, UGT2B7, SULT1C2 and DT-diaphorase (NQO1).

Kidney microsomes are supplied in 250 mM sucrose buffer. S9 and cytosolic fractions are supplied in 50 mM Tris-HCl, (pH 7.4 at 4°C), containing 150 mM KCl and 2 mM EDTA.

For specific kidney subcellular fraction characterization data, see ***appendix page 84***.

Kidney Human

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
H0610.R	Pooled Human Kidney Microsomes	Mixed	0.5 mL	10 mg/mL
H0610.RS9	Pooled Human Kidney S9 Fraction	Mixed	1.0 mL	5 mg/mL
H0610.RC	Pooled Human Kidney Cytosol	Mixed	1.0 mL	5 mg/mL

Kidney Monkey – Cynomolgus

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
P2000.R	Pooled Cynomolgus Monkey Kidney Microsomes	Male	0.5 mL	10 mg/mL
P2000.RS9	Pooled Cynomolgus Monkey Kidney S9 Fraction	Male	1.0 mL	5 mg/mL
P2000.RC	Pooled Cynomolgus Monkey Kidney Cytosol	Male	1.0 mL	5 mg/mL

Kidney Dog – Beagle

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
D1000.R	Pooled Beagle Dog Kidney Microsomes	Male	0.5 mL	10 mg/mL
D1000.RS9	Pooled Beagle Dog Kidney S9 Fraction	Male	1.0 mL	5 mg/mL
D1000.RC	Pooled Beagle Dog Kidney Cytosol	Male	1.0 mL	5 mg/mL

Kidney Rat – IGS Sprague-Dawley

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
R1000.R	Pooled IGS Sprague-Dawley Rat Kidney Microsomes	Male	0.5 mL	10 mg/mL
R1000.RS9	Pooled IGS Sprague-Dawley Rat Kidney S9 Fraction	Male	1.0 mL	5 mg/mL
R1000.RC	Pooled IGS Sprague-Dawley Rat Kidney Cytosol	Male	1.0 mL	5 mg/mL

Kidney Mouse – CD-1

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
M1000.R	Pooled CD-1 Mouse Kidney Microsomes	Male	0.5 mL	10 mg/mL
M1000.RS9	Pooled CD-1 Mouse Kidney S9 Fraction	Male	1.0 mL	5 mg/mL
M1000.RC	Pooled CD-1 Mouse Kidney Cytosol	Male	1.0 mL	5 mg/mL

Extrahepatic Subcellular Fractions

Intestine Subcellular Fractions

The use of intestinal subcellular fractions as a test system is highly recommended due to the importance of intestinal enzymes in the first-pass metabolism of orally ingested xenobiotics. Consequences of intestinal biotransformation can be very significant, including toxification by bioactivation and detoxification by aiding in excretion.

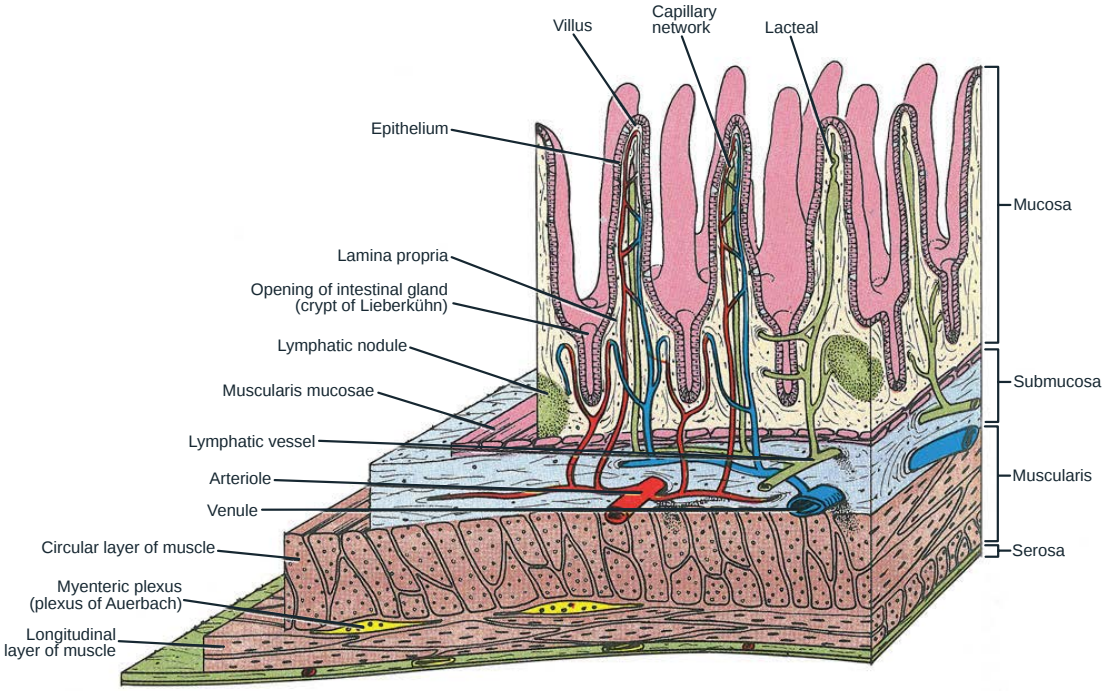
The majority of intestinal metabolic enzymes including cytochromes P450 and UGTs are contained in the mature enterocytes of the villus tips. To maintain the highest quality products, XenoTech uses a proprietary elution method to harvest only metabolically active enterocytes, yielding highly active subcellular fractions.

XenoTech offers intestinal subcellular fractions from five toxicologically significant species: human, Cynomolgus monkey, Beagle dog, Sprague-Dawley rat and CD-1 Mouse.

For metabolism studies focusing on the contribution of esterase, XenoTech now offers intestinal subcellular fractions prepared without PMSF (protease inhibitor) that yield high esterase activity from human, Beagle dog and Sprague-Dawley rat. PMSF-free intestine products are prepared using the same method as regular intestine products but do not include PMSF in the buffer.

Intestine microsomes are supplied in 250mM sucrose buffer. S9 and cytosolic fractions are supplied in a homogenization buffer which can be found in the ***appendix page 80***.

For specific intestine subcellular fraction characterization data, see ***appendix page 84***.



Intestine Subcellular Fractions *(cont.)*

Intestine Human

Product ID	Description	Gender	Volume	Concentration
H0610.I	Pooled Human Intestine Microsomes	Mixed	150 µL	10 mg/mL
H0610.IS9	Pooled Human Intestine S9 Fraction	Mixed	1.0 mL	4 mg/mL
H0610.IC	Pooled Human Intestine Cytosol	Mixed	1.0 mL	4 mg/mL
H0610.I(NP)	Pooled Human Intestine Microsomes, PMSF-free	Mixed	150 µL	10 mg/mL
H0610.IS9(NP)	Pooled Human Intestine S9 Fraction, PMSF-free	Mixed	1.0 mL	4 mg/mL
H0610.IC(NP)	Pooled Human Intestine Cytosol, PMSF-free	Mixed	1.0 mL	4 mg/mL

Intestine Monkey – Cynomolgus

Product ID	Description	Gender	Volume	Concentration
P2000.I	Pooled Cynomolgus Monkey Intestine Microsomes	Male	150 µL	10 mg/mL
P2000.IS9	Pooled Cynomolgus Monkey Intestine S9 Fraction	Male	1.0 mL	4 mg/mL
P2000.IC	Pooled Cynomolgus Monkey Intestine Cytosol	Male	1.0 mL	4 mg/mL
P2000.I(NP)	Pooled Cynomolgus Monkey Intestine Microsomes, PMSF-free	Male	150 µL	10 mg/mL
P2000.IS9(NP)	Pooled Cynomolgus Monkey Intestine S9 Fraction, PMSF-free	Male	1.0 mL	4 mg/mL
P2000.IC(NP)	Pooled Cynomolgus Monkey Intestine Cytosol, PMSF-free	Male	1.0 mL	4 mg/mL

Intestine Dog – Beagle

Product ID	Description	Gender	Volume	Concentration
D1000.I	Pooled Beagle Dog Intestine Microsomes	Male	150 µL	10 mg/mL
D1000.IS9	Pooled Beagle Dog Intestine S9 Fraction	Male	1.0 mL	4 mg/mL
D1000.IC	Pooled Beagle Dog Intestine Cytosol	Male	1.0 mL	4 mg/mL
D1000.I(NP)	Pooled Beagle Dog Intestine Microsomes, PMSF-free	Male	150 µL	10 mg/mL
D1000.IS9(NP)	Pooled Beagle Dog Intestine S9 Fraction, PMSF-free	Male	1.0 mL	4 mg/mL
D1000.IC(NP)	Pooled Beagle Dog Intestine Cytosol, PMSF-free	Male	1.0 mL	4 mg/mL

Intestine Rat – IGS Sprague-Dawley

Product ID	Description	Gender	Volume	Concentration
R1000.I	Pooled IGS Sprague-Dawley Rat Intestine Microsomes	Male	150 µL	10 mg/mL
R1000.IS9	Pooled IGS Sprague-Dawley Rat Intestine S9 Fraction	Male	1.0 mL	4 mg/mL
R1000.IC	Pooled IGS Sprague-Dawley Rat Intestine Cytosol	Male	1.0 mL	4 mg/mL
R1000.I(NP)	Pooled IGS Sprague-Dawley Rat Intestine Microsomes, PMSF-free	Male	150 µL	10 mg/mL
R1000.IS9(NP)	Pooled IGS Sprague-Dawley Rat Intestine S9 Fraction, PMSF-free	Male	1.0 mL	4 mg/mL
R1000.IC(NP)	Pooled IGS Sprague-Dawley Rat Intestine Cytosol, PMSF-free	Male	1.0 mL	4 mg/mL

Intestine Mouse – CD-1

Product ID	Description	Gender	Volume	Concentration
M1000.I	Pooled CD-1 Mouse Intestine Microsomes	Male	150 µL	10 mg/mL
M1000.IS9	Pooled CD-1 Mouse Intestine S9 Fraction	Male	1.0 mL	4 mg/mL
M1000.IC	Pooled CD-1 Mouse Intestine Cytosol	Male	1.0 mL	4 mg/mL
M1000.I(NP)	Pooled CD-1 Mouse Intestine Microsomes, PMSF-free	Male	150 µL	10 mg/mL
M1000.IS9(NP)	Pooled CD-1 Mouse Intestine S9 Fraction, PMSF-free	Male	1.0 mL	4 mg/mL
M1000.IC(NP)	Pooled CD-1 Mouse Intestine Cytosol, PMSF-free	Male	1.0 mL	4 mg/mL

Extrahepatic Subcellular Fractions

Skin Subcellular Fractions

Many drug metabolizing enzymes of the liver and other visceral organs are also expressed in the skin. The skin possesses a broad spectrum of xenobiotic metabolizing enzymes including cytochromes P450, flavin monooxygenases, esterases/amides, UDP-glucuronosyltransferases, glutathione *S*-transferases and sulfotransferases. Specific activities of these cutaneous enzymes are typically less than 10% of those found in the liver, yet may play a significant role in the biotransformation of xenobiotics absorbed and/or delivered through the skin.

Rat and mouse microsomes and S9 fractions are prepared from dorsal full-thickness skin tissue. Minipig and human S9 fractions are prepared from dermatome skin tissue.

Skin microsomes are supplied in 250 mM sucrose buffer. S9 and cytosolic fractions are supplied in 50 mM Tris-HCl, (pH 7.4 at 4°C), containing 150 mM KCl and 2 mM EDTA.

For specific skin subcellular fraction characterization data, see ***appendix page 85***.

Skin Human

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
H0610.ES9	Pooled Human Skin S9 Fraction	Mixed	0.5 mL	4 mg/mL

Skin Rat – IGS Sprague-Dawley

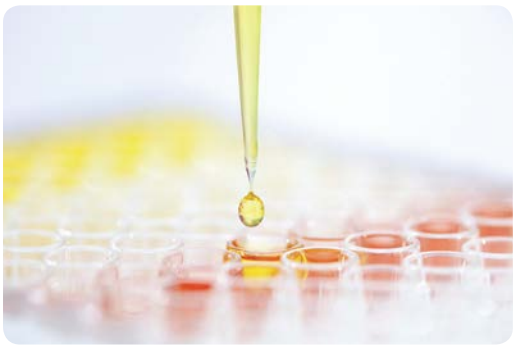
Product ID	*Description*	*Gender*	*Volume*	*Concentration*
R1000.E	Pooled IGS Sprague-Dawley Rat Skin Microsomes	Male	250 µL	10 mg/mL
R1000.ES9	Pooled IGS Sprague-Dawley Rat Skin S9 Fraction	Male	1.0 mL	5 mg/mL

Skin Mouse – CD-1

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
M1000.E	Pooled CD-1 Mouse Skin Microsomes	Male	250 µL	10 mg/mL
M1000.ES9	Pooled CD-1 Mouse Skin S9 Fraction	Male	1.0 mL	5 mg/mL

Skin Minipig – Gottingen

Product ID	*Description*	*Gender*	*Volume*	*Concentration*
Z0610.ES9	Gottingen Minipig Skin S9 Fraction	Mixed	0.5 mL	4 mg/mL



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Animal Liver Subcellular Fractions

Animal Liver Subcellular Fractions

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.

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

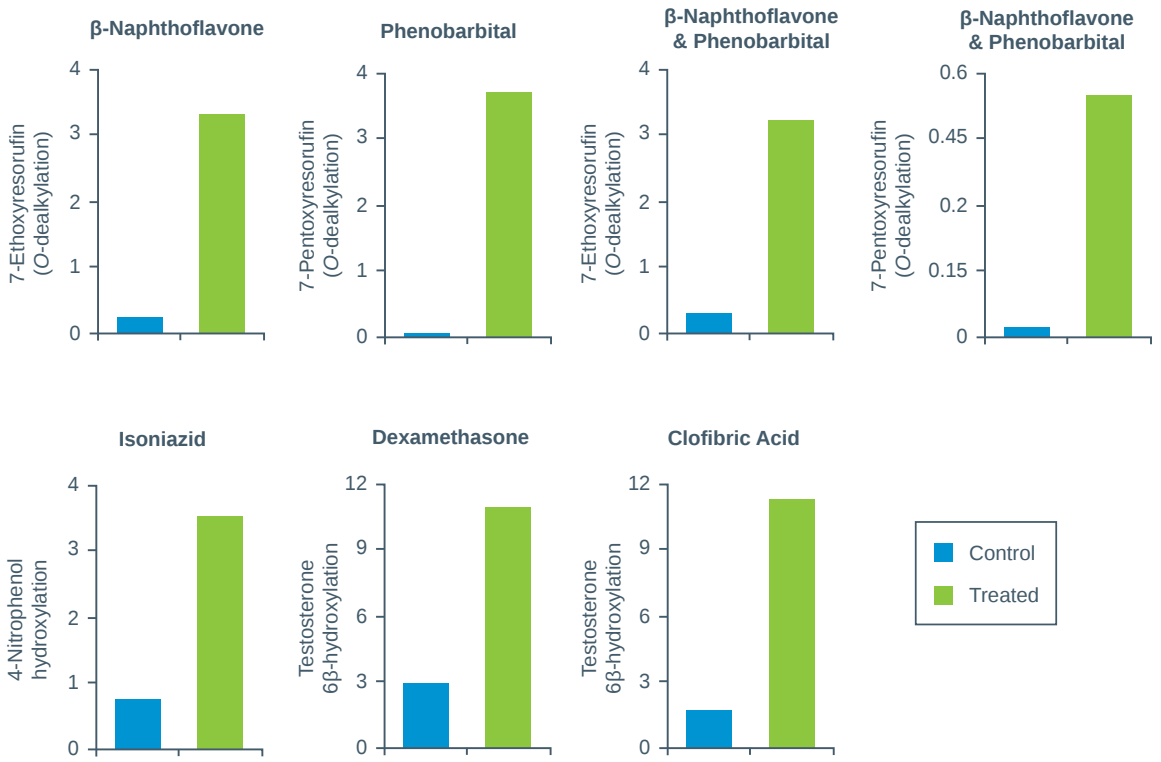
Product ID	Description	Gender	Volume	Concentration
M5000	Pooled C57BL/6 Mouse Liver Microsomes	Male	0.5 mL	20 mg/mL
M5000.S9	Pooled C57BL/6 Mouse Liver S9 Fraction	Male	1.0 mL	20 mg/mL
M5000.C	Pooled C57BL/6 Mouse Liver Cytosol	Male	1.0 mL	10 mg/mL

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. 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.

Ex vivo induction of CYP in rats treated with prototypical enzyme inducers



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

Treated Animal Liver Subcellular Fractions *(cont.)*

The following tables represent 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 Lysosomes	4	Mixed	0.25 mL
R0610.LT	Pooled IGS Sprague-Dawley Rat Liver Tritosomes	60	Mixed	0.25 mL

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**

RapidStart™ NADPH Regenerating System

rapidSTART™
NADPH Regenerating System

Add. Agitate. Activate!

NADPH is a cofactor which is required to support reactions catalyzed by drug metabolizing enzymes such as cytochrome P450 (CYP) and flavin-containing monooxygenases (FMO). Microsomes, S9 or Recombinant CYPs (Bactosomes) can be fortified with a NADPH regenerating system to support the long-term metabolism of drug/test articles by these enzymes. The RapidStart™ system maintains a suitable concentration of NADPH for 12 hours at room temperature and for 48 hours at 2-4°C.

The RapidStart™ system is packaged frozen in a single, durability-tested polycarbonate vial. This system consists of three pellets:

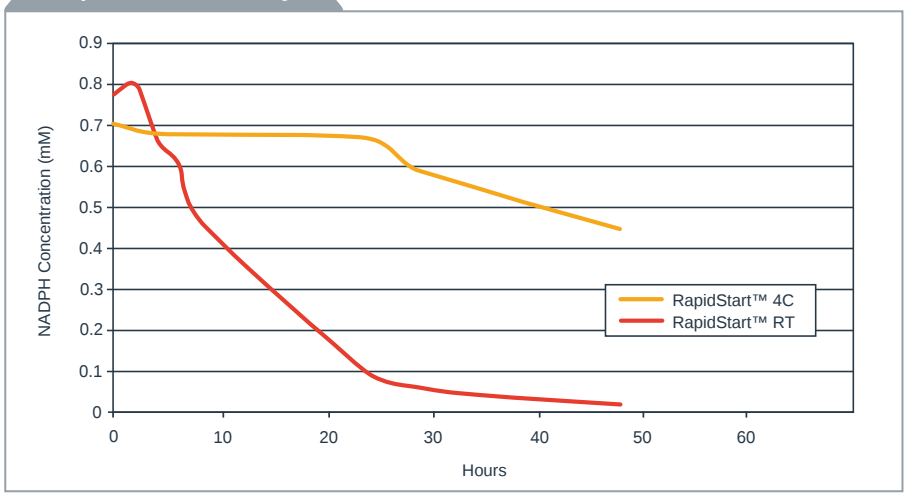
- NADP
- Glucose-6-phosphate dehydrogenase
- Glucose-6-phosphate

Preparation Procedure

Add: Add the appropriate amount of high purity water for the desired concentration[1]
Agitate: Vortex until pellets are fully dissolved
Activate: NADPH regenerating system has been activated and is ready for use.

Once activated, RapidStart™ maintains a suitable concentration of NADPH for 12 hours at room temperature or 48 hours at 2-4°C, allowing for multi-day use of the NADPH regenerating system.

RapidStart™ Stability



**4C denotes RapidStart's stability at 4°C; RT denotes RapidStart's stability at room temperature*

RapidStart™ NADPH Regenerating System

Product ID	*Description*	*Volume*
K5000	RapidStart™ NADPH Regenerating System	1.5 mL
K5000-10	RapidStart™ NADPH Regenerating System, 10-vial Kit	1.5 mL
K5100	RapidStart™ NADPH Regenerating System	0.3 mL
K5100-5	RapidStart™ NADPH Regenerating System, 5-vial Kit	0.3 mL

[1]*Dilution Concentration Tables on* ***appendix page 88***.

Custom Products

XenoTech's custom products division has over 20 years of experience preparing hepatocytes and subcellular fractions from a variety of species. All 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 XenoTech has prepared in the past and is not a complete list of XenoTech's capabilities for custom product preparations.

Species

- African Green Monkey
- B6C3F1 Mouse
- B6SJLF1 Mouse
- Balb/c Mouse
- Beagle Dog
- Brown Norway Rat
- C57BL/6 Mouse
- C57BL/6J Mouse
- Cat
- CD-1 Mouse
- Chicken
- Cow
- Cynomolgus Monkey
- Db/db Lean Mouse
- Db/db Obese Mouse
- Fischer 344 Rat
- Fox Chase SCID Mouse
- FVB Mouse
- GK Rat
- Goat
- Golden Syrian Hamster
- Gottingen Minipig
- Hanford Minipig
- Hartley Guinea Pig
- Horse
- Human (many demographics)
- Japanese White Rabbit
- KK.Cg.A_y/J Mouse
- KK/H1J Mouse
- Lewis Rat
- Long-Evans Rat
- Marmoset Monkey
- Mongolian Gerbil
- New Zealand Rabbit
- NU/NU Nude Mouse
- Ob/ob Lean Mouse
- Ob/ob Obese Mouse
- Owl Monkey
- Pig (standard)
- Rhesus Monkey
- SCID Mouse
- Sheep
- Swiss Webster Mouse
- Trout
- Wistar Han Rat
- Yucatan Minipig
- ZDF Lean Rat
- ZDF Obese Rat

Tissues

- Adrenal Gland
- Blood Plasma
- Brain
- Colon
- Duodenum
- Eye
- Heart
- Ileum
- Jejunum
- Kidney
- Liver
- Lung
- Muscle
- Ovary
- Pancreas
- Skin
- Spleen
- Stomach
- Testicle
- Etc.

Fractions

- Cytosol
- Hepatocytes
- Homogenate
- Lysosomes/Tritosomes
- Mast Cells
- Microsomes
- Mitochondria
- Pre-lysate
- S9 Fraction

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

Custom Products

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.

Recombinant Enzymes

XenoTech is a distributor of Cypex Recombinant Enzymes (Bactosomes) in North America. Cypex Bactosomes are a useful *in vitro* tool for drug metabolism, inhibition, reaction phenotyping and metabolite generation applications. Research has shown that under recommended conditions, *E. coli* expressed Bactosomes frequently exhibit greater activity than commercially available CYPs expressed from insect cells (Supersomes). All Cypex Bactosomes can be used in conjunction with the SimCYP simulator (SimCYP). Bactosomes are available expressing enzymes from a variety of species including human, monkey, dog, rat and mouse.

Classic Bactosomes

Cypex Bactosomes show excellent linearity over time, allowing longer incubations, generating better results. Reductase and High Reductase Bactosomes exhibit a limited linearity of substrate turnover with time. This is because that they contain high levels of NADPH P450 reductase, in turn creating higher activity. Low Reductase Bactosomes contain lower levels of NADPH P450 reductase, enhancing the linearity of substrate turnover with time making Bactosomes a perfect system for inhibition studies and more comparable to activities found *in vivo*.

More specific information on our full selection of Cypex Bactosomes, including Bactosome vs. Supersome comparisons, can be found on ***appendix pages 90-97***.

Bactosome Features:

- Patented *E. coli* expression system
- Excellent batch-to-batch consistency
- Robust P450 activity levels
- Extensive batch-specific data (K_m, V_{max}, linearity with time and enzyme, at K_m)
- Comprehensive selection, including modified-reductase choices and controls
- 10-30 minute linearity over time
- ISO9001:2009 compliant production and quality control

Bactosome Benefits:

- Reductase Bactosomes optimized for metabolism
- Low Reductase Bactosomes designed specifically for inhibition assays
- Utilize native P450 and better represent human enzymes
- Available with and without b_5
- Exhibit the best linearity over time compared to competitor rCYPs
- Least expensive commercial option

Custom CYPs and special packaging requests available. Contact us at **913.438.7450** for more information.

Human Classic Bactosomes

Enzyme	High Reductase	Low Reductase	Reductase + b_5	Low Reductase + b_5
1A1	CYP014	CYP018		
1A2	CYP001	CYP012		
1B1		CYP023		
1B1*3		CYP021		
1B1*4		CYP024		
2A6	CYP011		CYP064	
2A13	CYP033	CYP032		
2B6	CYP020	CYP016	CYP041	CYP061
2C8	CYP017	CYP047	CYP049	CYP052
2C9	CYP019	CYP006	CYP037	CYP038
2C9*2	CYP031		CYP042	
2C9*3	CYP039		CYP044	
2C18		CYP022		
2C19	CYP008	CYP028	CYP063	CYP062
2D6	CYP007	CYP013		
2D6*2	CYP027			
2D6*10	CYP029			
2D6*39	CYP030			
2E1	CYP009		CYP036	
2J2		CYP034		
3A4	CYP002	CYP010	CYP005	CYP035
3A5	CYP046	CYP015	CYP048	CYP045
3A7	CYP059	CYP053	CYP060	CYP054
4A11	CYP026	CYP025	CYP043	
4F2				CYP040
4F3B	CYP056	CYP055	CYP058	CYP057
17A1	CYP066	CYP065		
46A1	CYP068	CYP067		

**All Bactosomes are supplied at 1 nmol/vial*
**CYPxxx represents XenoTech's product ID*

Monkey Classic Bactosomes

Enzyme	Low Reductase	Type
2C20	CYP330	Cyno
2C43	CYP331	Cyno
2C75	CYP332	Cyno
2C76	CYP333	Cyno
3A8	CYP334	Cyno
3A5	CYP335	Cyno
1B1	CYP430	Rhesus

** All monkey CYPs are supplied at 1 nmol CYP per vial*
** Monkey CYP co-expressed with monkey NADPH-CYP reductase in E. coli*

Dog Classic Bactosomes

Enzyme	Low Reductase
1A1	CYP300
1A2	CYP307
1B1	CYP308
2B11	CYP301
2C21	CYP302
2C41	CYP303
2D15	CYP304
3A12	CYP305
3A26	CYP306

**All dog CYPs are supplied at 1 nmol CYP per vial*
** Dog CYP co-expressed with dog NADPH-CYP reductase in E. coli*

Recombinant Enzymes

Rat Classic Bactosomes

Enzyme	Low Reductase
1A2	CYP364
1B1	CYP363
2C11	CYP365
2D4	CYP361
2D18	CYP362
3A2	CYP366
3A9	CYP360

** All rat CYPs are supplied at 1 nmol CYP per vial*
** Rat CYP co-expressed with rat NADPH-CYP reductase in E. coli*

Mouse Classic Bactosomes

Enzyme	Low Reductase
1B1	CYP402
2B10	CYP400
3A11	CYP401

**All mouse CYPs are supplied at 1 nmol CYP per vial*
** Mouse CYP co-expressed with mouse NADPH-CYP reductase in E. coli*

Human Sulfotransferase Bactosomes

Sulfotransferase Enzyme	Low Reductase
1A1*1	CYP100
1A1*2	CYP105
1A2	CYP106
1A3	CYP101
1B1	CYP102
1C2	CYP107
1C4	CYP108
1E1	CYP103
2A1	CYP104

** Sulfotransferase Bactosomes are supplied at 1 nmol/vial at 1 mg/mL cytosolic protein*

New products are released on a regular basis. A current list of individual products and product details can be found on our website at **www.xenotech.com**

Control Bactosomes

Product ID	Description	Amount Per Vial
CYP003	Control Bactosomes	10 mg Protein
CYP004	Human Reductase Bactosomes	4 nmol Reductase
CYP099	Control Cytosol	1 mg Protein
CYP/EZ003	EasyCYP Control Bactosomes	5 mg Protein

** Control cytosol is supplied at a standard protein concentration of 10 mg/mL*

Other Human Enzymes

Product ID	Description	Amount Per Vial
CYP120	Human GSTA1	1 mg Cytosolic Protein
CYP121	Human GSTM1	1 mg Cytosolic Protein
CYP150	Human Aldehyde Oxidase	250 µL Cytosolic Extract
CYP151	Human Aldehyde Dehydrogenase 1A1	1 mg Cytosolic Protein
CYP152	Human Carboxylesterase 1 (CES1)	1 mg Membrane Protein
CYP153	Human Carboxylesterase 2 (CES2)	1 mg Membrane Protein
CYP200	Human UGT1A6	5 mg Membrane Protein

EasyCYP Bactosomes

Cypex EasyCYPs are the ultimate in convenience. They provide all the same features and benefits of the classic Bactosomes but EasyCYPs boast a standard CYP concentration, standard protein concentration and a standard vial size. EasyCYP Bactosomes are supplied at a CYP concentration of 1 nmol/mL, a protein concentration of 10 mg/mL and each vial contains 0.5 nmol of cytochrome P450.

For more information on EasyCYPs including incubation conditions, see ***appendix pages 90-97***.

Human EasyCYP Bactosomes

Enzyme	*High Reductase*	*Low Reductase*	*Reductase + b_5*	*Low Reductase + b_5*
1A1	CYP/EZ014	CYP/EZ018		
1A2	CYP/EZ001	CYP/EZ012		
1B1		CYP/EZ023		
1B1*3		CYP/EZ021		
1B1*4		CYP/EZ024		
2A6	CYP/EZ011		CYP/EZ064	
2A13	CYP/EZ033	CYP/EZ032		
2B6	CYP/EZ020	CYP/EZ016	CYP/EZ041	CYP/EZ061
2C8	CYP/EZ017	CYP/EZ047	CYP/EZ049	CYP/EZ052
2C9	CYP/EZ019	CYP/EZ006	CYP/EZ037	CYP/EZ038
2C9*2	CYP/EZ031		CYP/EZ042	
2C9*3	CYP/EZ039		CYP/EZ044	
2C18		CYP/EZ022		
2C19	CYP/EZ008	CYP/EZ028	CYP/EZ063	CYP/EZ062
2D6	CYP/EZ007	CYP/EZ013		
2D6*2	CYP/EZ027			
2D6*10	CYP/EZ029			
2D6*39	CYP/EZ030			
2E1	CYP/EZ009		CYP/EZ036	
2J2		CYP/EZ034		
3A4	CYP/EZ002	CYP/EZ010	CYP/EZ005	CYP/EZ035
3A5	CYP/EZ046	CYP/EZ015	CYP/EZ048	CYP/EZ045
3A7	CYP/EZ059		CYP/EZ060	
4A11	CYP/EZ026	CYP/EZ025	CYP/EZ043	
4F2				CYP/EZ040
4F3B	CYP/EZ056	CYP/EZ055	CYP/EZ058	CYP/EZ057
17A1	CYP/EZ066	CYP/EZ065		
46A1	CYP/EZ068	CYP/EZ067		

**All EasyCYPs are supplied at 0.5 nmol P450 at 1 nmol/mL, 10 mg/mL protein.*

Substrates & Metabolites

XenoTech is a North American distributor of Cypex's growing range of cytochrome P450 substrates and metabolites. Substrates are currently available for a number of P450s together with the corresponding metabolites, some of which are radio-labeled to ease detection. All of the compounds are supplied at high purity and in convenient package sizes.

Cytochrome P450 Substrates and Metabolites

Product ID	*Product*	*Description*	*Volume*
CYP500	4′-hydroxydiclofenac	CYP2C9 metabolite of diclofenac	0.1 mg
CYP500-5	4′-hydroxydiclofenac	CYP2C9 metabolite of diclofenac	5 mg
CYP501	6α-hydroxypaclitaxel	CYP2C8 metabolite of paclitaxel	0.1 mg
CYP501-5	6α-hydroxypaclitaxel	CYP2C8 metabolite of paclitaxel	5 mg
CYP502	[1-^{14}C] lauric acid	Substrate for CYP2E1 and CYP4A11. Supplied as a solid	50 µCi
CYP503	[1-^{14}C] 11-hydroxylauric acid	CYP2E1 metabolite of lauric acid (minor metabolite of CYP4A11). Supplied as a solid	50 µCi
CYP504	[1-^{14}C] 12-hydroxylauric acid	CYP4A11 metabolite of lauric acid. Supplied as a solid	50 µCi
CYP505	Tolbutamide	Substrate for CYP2C	100 mg
CYP506	Hydroxytolbutamide	CYP2C metabolite of tolbutamide	10 mg
CYP507	Carboxytolbutamide	Metabolite of tolbutamide	10 mg
CYP508	Carboxytolbutamide methyl ester	Derivative of carboxytolbutamide, a metabolite of tolbutamide	10 mg
CYP509	Tolbutamide kit	Kit consisting of one each of CYP505-CYP508	
CYP510	7-ethoxyresorufin	Red solid. Substrate for CYP1A1, CYP1A2 and CYP1B1	10 mg
CYP511	Resorufin	CYP1A1, CYP1A2 and CYP1B1 metabolite of 7-ethoxyresorufin	100 mg
CYP512	7-benzyloxyquinoline (7BQ)	White solid. Packaged under nitrogen. Substrate for CYP3A4	10 mg
CYP513	7-hydroxyquinoline (7HQ)	Off-white solid. Packaged under nitrogen. Metabolite of 7-BQ	10 mg
CYP514	[3-^{14}C] 7-ethoxycoumarin	CYP substrate. Supplied as a solid	50 µCi
CYP515-10	7-methoxy-4-aminomethylcoumarin (MAMC)	White solid. Packaged under nitrogen. Substrate for CYP2D6	10 mg
CYP515-25	7-methoxy-4-aminomethylcoumarin (MAMC)	White solid. Packaged under nitrogen. Substrate for CYP2D6	25 mg
CYP516	7-hydroxy-4-aminomethylcoumarin (HAMC)	White solid. Packaged under nitrogen. CYP2D6 metabolite of MAMC	10 mg
CYP517-10	7-methoxy-4-trifluoromethylcoumarin (MFC)	White solid. Packaged under nitrogen. Substrate for CYP2C9	10 mg
CYP517-25	7-methoxy-4-trifluoromethylcoumarin (MFC)	White solid. Packaged under nitrogen. Substrate for CYP2C9	25 mg
CYP519	7-hydroxy-4-trifluoromethylcoumarin (HFC)	White solid. Packaged under nitrogen. Metabolite of MFC and BFC	10 mg
CYP520	Omeprazole	White solid. Packaged under nitrogen. Substrate of CYP2C19	1 g
CYP521-10	3-*O*-methylfluorescein	Pale yellow solid. Packaged under nitrogen. Substrate of CYP1A1 and CYP2C19	10 mg
CYP521-25	3-*O*-methylfluorescein	Pale yellow solid. Packaged under nitrogen. Substrate of CYP1A1 and CYP2C19	25 mg
CYP522-10	Tienilic acid	White solid. Packaged under nitrogen. Mechanism-based inhibitor of CYP2C9	10 mg

Cytochrome P450 Substrates and Metabolites *(cont.)*

Product ID	*Product*	*Description*	*Volume*
CYP522-25	Tienilic acid	White solid. Packaged under nitrogen. Mechanism-based inhibitor of CYP2C9	25 mg
CYP523-25	Paclitaxel	White Solid. Packaged under nitrogen. Substrate of CYP2C8	25 mg
CYP523-100	Paclitaxel	White Solid. Packaged under nitrogen. Substrate of CYP2C8	100 mg
CYP524-100	Ketoconazole	White solid. Packaged under nitrogen. CYP3A4 inhibitor	100 mg
CYP524-1000	Ketoconazole	White solid. Packaged under nitrogen. CYP3A4 inhibitor	1 g
CYP525	Diclofenac	White solid. Packaged under nitrogen. Substrate of CYP2C9 and CYP2C18	10 g
CYP526	Amodiaquine dihydrochloride dihydrate	Yellow solid. Substrate of CYP2C8	5 g
CYP527-10	Desethylamodiaquine	Solid. CYP2C8 metabolite of amodiaquine	10 mg
CYP527-25	Desethylamodiaquine	Solid. CYP2C8 metabolite of amodiaquine	25 mg
CYP528	Terfenadine	White solid. Substrate of CYP3A4 and CYP2J2	5 g
CYP529-10	(-)-*N*-3-benzylphenobarbital	White solid. Specific inhibitor of CYP2C19	10 mg
CYP529-25	(-)-*N*-3-benzylphenobarbital	White solid. Specific inhibitor of CYP2C19	25 mg
CYP530-10	Montelukast sodium	Off-white solid. Inhibitor of CYP2C8	10 mg
CYP530-25	Montelukast sodium	Off-white solid. Inhibitor of CYP2C8	25 mg
CYP531-10	Diethoxyfluorescein (DEF)	Pale yellow or cream solid. Packaged under argon. Substrate of CYP2C8 and CYP3A4	10 mg
CYP531-25	Diethoxyfluorescein (DEF)	Pale yellow or cream solid. Packaged under argon. Substrate of CYP2C8 and CYP3A4	25 mg
CYP532	*S*-mephenytoin	White or off-white solid. Packaged under argon. Substrate of CYP2B6 and CYP2C19	5 mg
CYP533	(+/-)-4′-hydroxymephenytoin	White, off-white, or light yellow solid. Packaged under argon. CYP2C19 metabolite of mephenytoin	5 mg

**All above substrates and metabolites are intended for laboratory (research) purposes only. Not intended for drug or human use.*

New products are released on a regular basis. A current list of individual products and product details can be found on our website at **www.xenotech.com**

Appendix: Cellular Products

Hepatocytes

Isolation of Hepatocytes from Fresh Liver Tissue

Hepatocytes are isolated from fresh tissue either through *in situ* perfusion (for rodent species) or excised whole fresh tissue (human and other non-rodent species) using a two-step collagenase digestion process. Isolated hepatocytes are washed through multiple centrifugation steps and then plated for fresh culture, resuspended in storage media for fresh suspensions or cryopreserved using a step-wise freezing method to minimize cryopreservation injury.

Preparation of Fresh Plated Hepatocytes

Freshly isolated hepatocytes can be maintained in culture for up to 7 days. Plated hepatocytes are an ideal test system for measurement of induction of a given compound or long term stability experiments requiring time points of 24, 48 or 72-hours in culture. XenoTech uses freshly isolated hepatocytes (viability >70%) and seeds the hepatocytes on Collagen I coated culture vessels (6-well, 12-well, 24-well, 48-well and 96-well), hepatocytes are allowed to attach for a period of 2-4 hours then overlayed with a serum-free media containing Matrigel to complete the sandwich culture configuration. Cultures are then fed daily and observed for morphology and confluency.

Hepatocyte Incubations – Suspension

Cryopreserved or freshly suspended hepatocytes are ideal test systems for studying drug metabolism, drug inhibition, compound stability, transporter uptake, etc. where short-term incubation times are needed (up to 4 hours). Typical incubation conditions for using hepatocytes in suspension are shown below.

Incubation Vessel	Flat-bottomed uncoated culture dishes (any well format)
Cell Concentration	1 million cells per mL
Incubation Media	Opti**Incubate**, Krebs Henseleit Buffer or similar
Incubator	37°C, humidified chamber
Incubation Times	0 (serve as blanks) 30, 60, 90, 120, 240 min

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

CYP	*Substrate*	*Substrate Concentration[1] (µM)*	*Substrate Solvent (final % v/v)*	*Metabolite Standards*
Various	7-Ethoxycoumarin	50	Methanol (0.5%)	Varies depending on metabolite
Phase II	7-Hydroxycoumarin	100	Methanol (0.1%)	Varies depending on metabolite
CYP1A2	Phenacetin	100	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
CYP3A4/5	Testosterone	250	Methanol/Acetonitrile (1%/0.5%)	10-2000 ng/mL β-Hydroxytestosterone
CYP3A4/5	Midazolam	30	Methanol (1%)	1-200 ng/mL 1-Hydroxymidazolam

[1]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
Testosterone	6β-Hydroxytestosterone-d2
Midazolam	α-Hydroxymidazolam-d3

Transporter uptake incubation conditions are similar to those listed above. They are carried out in KHB with a short incubation time (1 minute) in an effort to capture a rate near the linear phase of uptake. Additionally, incubations are performed at 4°C and 37°C to allow the differentiation of active and passive transport. The oil spin method is then used to stop incubations by separating the cells from the substrate/test compound.

Uptake Transporter	*Substrate*	*Concentration*[1]
OATP1B1	Esterone sulfate	1 µM
OATP1B3	Cholecystokinin-8	1 µM
OCT1	1-Methyl-4-phenylpyridinium Iodine	1 µM
NTCP	Taurocholic Acid	1 µM

[1]Concentration is a combination of “cold” and radio-labeled compound.

Appendix: Hepatocytes

Hepatocyte Incubations – Plated

Cryopreserved (plateable human or animal) or freshly plated hepatocytes are ideal test systems for studying drug metabolism (where long-term time points are needed), enzyme induction, and transporter uptake. When using cryoplateable lots of hepatocytes it is important to follow each step of the thawing and plating protocol, including using the recommended cell seeding concentration provided on each data sheet. This will help to ensure the best culture possible. Once hepatocytes are thawed and seeded on culture dishes at the appropriate cell concentration, they should be allowed an attachment period of 4 hours (or 24 hours for rat) in a 37°C, humidified incubator with 95%/5% (air/CO_2). After the attachment period, gently agitate the culture plates and aspirate media/unattached cells then follow with a solution of ice-cold culture media (K8300 Opti**Culture**) containing 0.25 mg/mL of Matrigel (or equivalent). This will complete the sandwich culture configuration. Cultures should be returned to the incubator and fed or dosed with compound every 24 hours. Recommended seeding concentrations are listed on the particular lot's data sheet (refer to plating protocol for seeding volumes).

Induction Use

Once the hepatocyte culture is deemed having good morphology and confluency (usually 1-2 days after plating), dosing of the cultures can be initiated. Typically, cells are dosed every 24 hours using culture media (K8300 Opti**Culture**) containing compound dissolved in appropriate solvent, DMSO or Saline. It is best to keep the vehicle solvent at a concentration not exceeding 0.1% in the dosing media.To measure induction and/or toxicity, it is important to set aside some of the culture wells for a control, being treated with only the vehicle solvent and culture media. Treatment typically lasts 2-3 days; induction can then be measured using *in situ* incubations by adding substrate (in media) directly to the culture or by harvesting of mRNA. Below is an example of typical incubation conditions XenoTech uses to characterize plateable human hepatocytes for induction using enzymatic activity:

Prototypical Inducers for CYP1A2, 2B6, 3A4	Vehicle control 50 µM Omeprazole 750 µM Phenobarbital 10 µM Rifampin
Culture Plate/Incubation Vessel	24-well plate (dosed in triplicate)
Marker Substrates	Phenacetin (1A2) – 100 µM Buproprion (2B6) – 100 µM Testosterone (3A4) – 250 µM
Incubation Volume	330 µL (Culture media containing substrate)
Incubation Time	30 min

Metabolism Use

Once the hepatocytes have had a chance to attach to the culture vessel (approximately 4 hours), metabolism experiments can be initiated. Test compound should be dissolved in the appropriate vehicle solvent, typically DMSO, methanol or equivalent. It is important to ensure final solvent concentration does not exceed 0.1%. Incubation times can vary based on experimental design, 4 hrs out to 72 hrs. The cells will maintain in culture 5 days and possibly up to 7. Typically at each time point a volume of media is removed from the culture and quenched with organic solvent, or organic solvent can be directly added to the culture to quench the incubations. In both scenarios, it is recommended to centrifuge the vessels to remove precipitated protein prior to analysis.

Tips & Techniques for Thawing/Using Hepatocytes

Thawing hepatocytes is a simple process that requires a few important techniques to optimize the preparation.

- Cryopreserved hepatocytes should remain stored in LN_2 vapor phase until use. When ready to thaw the cells, rapidly transfer the vials to a 37°C water bath. The cells become unstable at temperatures of -80°C and warmer.
- It is crucial to NOT overthaw the hepatocytes. Overthawing will result in decreased yield and viability. For non-CryostaX hepatocyte products, thawing should take roughly 80 seconds in a 37°C water bath. Invert the vial, if the frozen cell pellet falls to the lid it should be immediately transferred into the Opti**Thaw** media. For CryostaX products, the pellets should be immediately transferred into *pre-warmed* Opti**Thaw** media, no thawing required.
- For best results ensure level of water in the water bath is above the highest frozen point in the vial.
- Ensure that the Opti**Thaw** media is *pre-warmed* to ~37°C prior to starting the thaw process.
- For suspension incubations, dispense all substrate solutions and set up incubation vessels *prior* to thawing hepatocytes. While suspended hepatocytes are stable at room temperature, viability drops over time.
- To obtain maximum yield, remember to rinse the cryo vial with media after removing the cell pellet.
- Never vortex or vigorously resuspend the hepatocytes. A gentle rocking motion is recommended.
- When aspirating supernatant, keep the tip of the aspirator at the highest level of media to ensure any cell debris is removed before reaching the viable cell pellet.
- XenoTech does not recommend pouring off supernatant, due to the high risk of losing the viable cell pellet during the pour process.
- Automated cell counters are not recommended for hepatocytes due to various inaccuracies of different models. We recommend performing two Trypan blue counts after centrifugation for verification of yield and viability.
- One hepatocyte isolation kit can be used to thaw up to 3 vials.

Our online Resource Library offers easy access to a variety of trusted information sources. Access it at **www.xenotech.com/knowledge**

Appendix: Hepatocytes

Protocol for Thawing Cryopreserved Hepatocytes

The following procedure may be carried out in a biosafety containment hood to reduce the risk of contamination and also minimize contact with potentially biohazardous material.

Step-wise Procedure for Thawing Hepatocytes (K8000, K8100 & K8500 OptiThaw)

This procedure describes the steps required for the thawing of hepatocytes using XenoTech's Hepatocyte Thawing Media.

1 Warm the Opti**Thaw** media to 37 ± 1°C in a water bath before use (typically takes ~15-20 minutes).

2 Remove the cryotube from the LN_2 storage unit and immediately place in a 37 ± 1°C water bath for ~80 seconds until the frozen cell pellet can move freely when the cryotube is inverted. *Do not over-thaw.*

*Note: For CryostaX™ pools, the contents of the vial can be immediately dispensed into pre-warmed Opti**Thaw** media. Do not thaw CryostaX™ in a water bath. Once contents are transferred into Opti**Thaw**, gently invert the tube until all of the pellets have melted.*

3 Transfer the frozen pellet from the cryotube into Opti**Thaw** media. Rinse each cryotube with about 1.5 mL of Opti**Thaw**. Pour this rinse back into the Opti**Thaw** tube and gently invert until fully melted.

4 Centrifuge at 100 *x* g for 5 minutes at room temperature or 2-8°C.

– Aspirate and discard the supernatant fluid without disturbing the cell pellet.

5 Resuspend the cell pellet(s) with Opti**Plate** media (for plating hepatocytes) or Opti**Incubate** (for suspension incubations). Be careful to not over-dilute the cells based on final target cell concentration.

– **DO NOT VORTEX.** Remove 50 µL of the homogenous cell suspension and dispense the 50 µL aliquot into the Opti**Count** tube.

– Mix gently. Cell viability can now be assessed by placing an aliquot from the counting tube on a hemacytometer and counting the dead (blue) cells and viable cell number.

6 Measure the volume of the cell suspension and *q.s.* to the desired concentration.

Protocol for Suspension Incubations

Materials Needed:

- Flat-bottomed, uncoated incubation vessel.
- Static or shaking incubator temperature: 37°C, Atmosphere: Rh (95%), CO2 (5%), for long-term incubations a humidified incubator is recommended.
- Opti**Incubate** Hepatocyte Media (K8400)

Typical final cell concentration per incubation	**1 x 106 cells per mL**
Flat-bottom incubation vessel	**6-, 12-, 24-, 48-, 96- well**
Typical incubation times	**0, 30, 60, 90, 120, 240 min**

Incubation Media Selection:

For long-term incubations (> 2 hours), Opti**Incubate** Hepatocyte Media (K8400) is recommended to maintain a higher viability throughout the incubation time.

If the test compound may be susceptible to protein binding, a buffer such as KHB is recommended because it does not contain any protein supplementation.

Krebs Henseleit Buffer (KHB)
pH 7.4 *no nutritional supplementation* (Sigma)

Cryopreserved Hepatocyte Sample Preparation Worksheet

This worksheet may be used to record information during the preparation of your hepatocyte sample. Prepare additional copies of this sheet as needed.

Hepatocyte Sample Identification

# Vials Thawed	
Sample ID (Species/Lot Number)	**1.5 mL**

Date of hepatocyte isolation: _____

Trypan Blue Cell Count Analysis

A trypan blue exclusion analysis should be performed (step 5 in the thawing protocol) following re-suspension of the initial cell pellet.

Cells Counted		% Viability [A/(A+B)] x 100	Dilution factor[1]	Hemacytometer factor[2]	Volume of sample[3]	Number of viable hepatocytes[4]	Final cell concentration[5]
Live	Dead						
A	B		C	D	E		
				10,000			
				10,000			

1. The dilution factor will equal 10 if a 50 µL aliquot of the cell suspension was dispensed into XenoTech's hepatocyte isolation Opti**Count** tube for subsequent counting. If an alternate means of dilution was used, a different dilution factor should be calculated.
2. The hemacytometer factor will typically equal 10,000. For more information consult your hemacytometer manufacturer.
3. Volume of the sample indicates the total volume of the cell suspension from which the counting aliquot was removed.
4. The number of viable hepatocytes may be calculated from the following equation:

   $\left(\frac{A}{\text{quadrants}}\right) \times C \times D \times E$ where "quadrants" equals the number of quadrants counted on the hemacytometer.

5. The desired concentration should be determined based on the specific requirements of your experimental design.

Sample Dilution

Use the following table to calculate the final volume needed to reach the desired cell concentration:

# of viable hepatocytes (determined above)	Desired cell concentration for use	Final volume	Volume of media to add to reach desired conc.
F	G	H	I

H = F/G I = H - Volume of sample

Appendix: Hepatocytes

Protocol for Plating and Culturing Hepatocytes

Materials Needed:

- BioCoat™ Collagen I Cellware (BD Biosciences) or equivalent
- Cell Culture Incubator Temperature: 37°C Atmosphere: Rh (95%), CO_2 (5%)
- Opti**Culture** Media and Pen/Strep (K8300)*
- Opti**Plate** Hepatocyte Media (K8200)
- Opti**Matrix** Hepatocyte Overlay (K8600/K8650)

**K8300 comes with a supplemental vial of Pen/Strep. Add the entire contents of the vial to the bottle of media and update the expiration date to one month from the date of the addition.*

Procedure

1 Thaw hepatocytes as stated previously.

2 Dilute the hepatocyte suspension to the desired concentration with Opti**Plate** media. The table below shows a range of recommended seeding densities for each species. See the lot specific data sheet for a particular lot's recommended seeding density.

3 Add appropriate volume of cell suspension to each well. The table below provides recommended seeding volumes for the various species and plating formats.

Species	6-Well Format		12-Well Format		24-Well Format		48-Well Format		96-Well Format*	
	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well	Recommended Seeding Density (million cells/mL)	Recommended Seeding/Feeding Volume Per Well
Human	1.0 - 1.6	1.7 mL	1.0 - 1.6	650 µL	1.0 - 1.6	330 µL	0.75	200 µL	0.75	75 µL
Rat	1.2 - 1.4	1.7 mL	1.2 - 1.4	650 µL	1.2 - 1.4	330 µL	1.2 - 1.4	150 µL	0.6 - 0.7	75 µL
Monkey	1.4 - 2.2	1.7 mL	1.4 - 2.2	650 µL	1.4 - 2.2	330 µL	1.4 - 2.2	150 µL	1.4 - 2.2	50 µL
Mouse	0.4 - 0.6	1.7 mL	0.4 - 0.6	650 µL	0.4 - 0.6	330 µL	0.4 - 0.6	150 µL	0.4 - 0.6	50 µL

** Do not swirl 96-well plates to distribute cells.*

4 Place the seeded culture vessel in the 37°C incubator and swirl in a figure 8 pattern to evenly distribute the cell suspension. *Do not swirl 96-well plates.*

5 Allow cells to attach for 2-4 hours in a 37°C humidified CO_2 static incubator. Check attachment every hour until sufficient confluency is achieved (the hepatocytes will flatten out over time to fill in the majority of the gaps).

6 After the attachment period, swirl the culture vessel (to suspend the unattached cells) and aspirate media containing non-attached cells.

7 Add appropriate volume of 2-8°C Opti**Culture** Media solution (with or without Opti**Matrix**) to each well or plate and return dishes to incubator.

- Opti**Matrix**, as used for overlay, should be diluted to 0.25 mg/mL in the Opti**Culture** Media.
- To achieve maximum confluency when working with rat hepatocytes, DO NOT include Opti**Matrix** in the Hepatocyte Culture Media at the 2-4 hour time point media change. Opti**Matrix** overlay should be performed 18-24 hours post seeding.

8 Every 24 hours, the media should be aspirated and replaced with Opti**Culture** Media warmed to 37°C.

9 Dosing with compound can begin after the hepatocytes have been in culture for 24-48 hours.

- Media should be aspirated and replaced with fresh dosing solution (Opti**Culture** Media and test compound) at 37°C every 24 hours.
- Cultures can be maintained for 6 to 7 days.

Cryopreserved Human Hepatocytes		CryostaX™ Pooled	CryostaX™ Plateable Pooled	HepatoSure™ Pooled	Cryoplateable	Suspension	Uptake Transporter Characterized	Geneknown™
Product ID		HPCH10 HPCH20-50	HPCH05+ HPCH10+	HCP100.H15	H1000.H15B+ H1000.H15C+ H1500.H15B+ H1500.H15C+	H1000.H15-3 H1000.H15B H1000.H15C H1500.H15-3 H1500.H15B H1500.H15C	H1000.H15T H1500.H15T	Varies. See **page 12** for specific product IDs.
Gender		Mixed	Mixed	Mixed	Gender-Specific	Gender-Specific	Gender-Specific	Mixed
Volume/Vial		1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	0.9 mL
Pool Size		10-20 Donors	5-10 Donors	100 Donors	Single Donor	Single Donor	Single Donor	3
Assured Minimum Yield (AMY)		5 million cells	5 million cells	5 million cells	4-6 million cells	3-6 million cells	4-6 million cells	4.5 million cells
Characterization Pro								
Enzyme	**Marker Substrate Reaction**							
CYP1A2	Phenacetin *O*-dealkylation	✓	✓	✓	✓	✓	✓	✓
CYP2A6	Coumarin 7-hydroxylation	✓		✓	✓	✓	✓	✓
CYP2B6	Bupropion hydroxylation	✓	✓	✓	✓	✓	✓	✓
CYP2C8	Amodiaquine *N*-dealkylation	✓	✓	✓	✓	✓	✓	✓
CYP2C8/9	Tolbutamide methyl-hydroxylation							
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	✓	✓	✓	✓	✓	✓	✓
UGT	7-Hydroxycoumarin glucuronidation	✓	✓	✓	✓	✓	✓	
SULT	7-Hydroxycoumarin sulfonation	✓	✓	✓	✓	✓	✓	
Transporter Characte								
OCT1	1-Methyl-4-phenylpyridinium Iodine	✓	✓	✓			✓	
OATP1B1	Estrone-3-sulfate	✓	✓	✓			✓	
OATP1B3	Cholecystokinin-8	✓	✓	✓			✓	
NTCP	Taurocholic Acid	✓	✓	✓			✓	
Induction Data								
CYP1A2	Omeprazole				✓			
CYP2B6	Phenobarbital				✓			
CYP3A4	Rifampin				✓			
Additional Data								
Intrinsic Clearance Data				✓				
Aldehyde Oxidase Activity				✓				
Isolation Viability		✓	✓	✓	✓	✓	✓	
Donor Genotype								✓

Appendix: Human Hepatocytes

Fresh Human Hepatocytes

		Plated	*Suspension*
	Product ID	HHP06M HHP12M HHP24M HHP48M HHP96M	HHS01
	Gender	Gender-Specific	Gender-Specific
	Format	6-96 well	Vial
Characterization Provided			
Enzyme	***Marker Substrate Reaction***	***Fresh***	***Suspension***
CYP	7-Ethoxycoumarin *O*-dealkylation		✓
UGT	7-Hydroxycoumarin glucuronidation		✓
SULT	7-Hydroxycoumarin sulfonation		✓
Induction Data			
Enzyme	***Marker Substrate Reaction***	***Fresh***	***Suspension***
CYP1A2	Phenacetin *O*-dealkylation	✓	
CYP2B6	Bupropion hydroxylation	✓	
CYP3A4	Midazolam 1′-hydroxylation	✓	
Additional Data			
Isolation Viability		✓	✓

Due to the time-sensitive nature of fresh hepatocytes, customers may sign up for **Liver Alert** emails. These email notifications are sent out when fresh human hepatocytes become available for purchase. Visit **xenotech.com/liver-alert** to sign up or contact our customer service office at **913.GET.P450**.

Appendix: Animal Hepatocytes

Cryopreserved Animal Hepatocytes

		Cryoplateable	*Pooled*
	Standard Species Available	Monkey Rat Mouse Dog	Monkey Minipig Dog Rabbit Rat Mouse
	Gender	Gender-Specific	Gender-Specific
	Format	Vial	Vial
	Assured Minimum Yield (AMY)	2 - 7 million cells	2 - 7 million cells
Characterization Provided			
Enzyme	***Marker Substrate Reaction***	***Plateable***	***Pooled***
CYP	7-Ethoxycoumarin *O*-dealkylation	✓	✓
UGT	7-Hydroxycoumarin glucuronidation	✓	✓
SULT	7-Hydroxycoumarin sulfonation	✓	✓
Additional Data			
Isolation Viability		✓	✓

Fresh Animal Hepatocytes

		Fresh
	Standard Species Available	Rat Mouse
	Gender	Gender-Specific
	Format	6-96 well
Characterization Provided		
Enzyme	***Marker Substrate Reaction***	***Plateable***
CYP	7-Ethoxycoumarin *O*-dealkylation	✓
UGT	7-Hydroxycoumarin glucuronidation	✓
SULT	7-Hydroxycoumarin sulfonation	✓
Additional Data		
Isolation Viability		✓

Appendix: Kupffer Cells

Activation of Human Kupffer Cells by Bacterial Lipopolysaccharide

Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) are two of the cytokines released by activated Kupffer cells, which function as regulators of multiple cellular processes including an inflammation and response to an infection. In that capacity they contribute to regulation of drug metabolizing enzymes such as CYPs that are known to be suppressed during an inflammation, an infection or a co-administration with certain therapeutic proteins. Bacterial endotoxin (lipopolysaccharide, LPS) activates Kupffer cells and is commonly use to examine macrophages' functionality *in vitro*.

To measure changes in the abundance of TNF-α and IL-6 cultures of human Kupffer cells are cultured in medium supplemented with fetal bovine serum, Pen/Strep and insulin. Cell culture incubator atmosphere phere is maintained as 5% CO_2-95% air and 95% relative humidity at 37°C. Macrophages are stimulated with 5 or 50 µg/mL LPS (Sigma-Aldrich, St. Louis, MO) in medium for 24 h. To quantitate cytokine's mRNA expression total RNA is extracted from cells lysed in TRIzol reagent followed by purification with the RNeasy Mini Kit (Qiagen, Valencia, CA). Purified RNA is reverse transcribed to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) and the Applied Biosystems 7900HT Real-Time PCR System. IL-6 and TNF-α primers and probes are purchased from Applied Biosystems. Relative quantification based on GAPDH use as a calibrator, is calculated with RQ Manager Software. To measure cytokine protein levels enzyme-linked immunosorbent assays (ELISA) assay kits for IL-6 and TNF-α are purchased from AssayMax (St. Charles, MO) and used according to the manufacturer's instructions. Colorimetric measurements are conducted with the Synergy HT microplate reader (Biotek, Winooski, VT).

Tips for Working with Kupffer Cells

- It is important to keep all reagents at 2-8°C during the thawing process. Kupffer cells will adhere to any substrate if the media temperature exceeds 20°C.
- Thaw time is critical; overthawing cryopreserved Kupffer cells will result in low yield and viability. Solid ice pellet should be dumped directly into the 15 mL conical containing 9 mL of cold media.
- For best results ensure level of water in the water bath is above the highest frozen point in the vial.
- When aspiring supernatant, keep tip of the aspirator at the highest level of media to ensure any cell debris is removed before reaching the viable cell pellet.
- XenoTech does not recommend pouring off supernatant, due to the high risk for losing the viable cell pellet during the pour process.
- Never vortex or vigorously re-suspend the Kupffer cells.
- We recommend performing two Trypan blue counts after the spin for verification of yield and viability.
- Although cells begin to attach to the plate surface at day 5, higher confluency will be reached if the cells are allowed to attach for longer periods of time.

Protocol for Thawing and Culturing Cryopreserved Kupffer Cells

The following procedure should be carried out in a biosafety containment hood to reduce the risk of contamination and also minimize contact with potentially biohazardous material.

Step-wise Procedure for Thawing Kit (K8700 Opti**Thaw**)

This procedure describes the steps required for the isolation of Kupffer cells using XenoTech's Kupffer Cell Thawing / Culture Media.

Materials Needed:

- K8700 Opti**Thaw** - note this media will be used for thawing and culturing Kupffer Cells
- Trypan Blue solution, 0.4%
- 24-well cell culture-treated plate

1 Aliquot 9 mL of cold thawing media (Opti**Thaw**) into a 15 mL conical tube and place on ice.

2 Remove the cryo vial from the LN_2 storage unit and immediately place in a 37 ± 1°C water bath for ~80 seconds. The frozen cell pellet should move freely when the cryo vial is inverted. *Do not overthaw.*

3 Dump the frozen pellet from the cryo vial into the 15 mL conical tube containing 9 mL of media. Rinse each cryo vial with 1.0 mL of cold media. Pour this rinse into the 15 mL conical tube. Gently invert the 15 mL conical tube until all ice is melted.

4 Centrifuge the cells at 500 x *g* for 5 minutes at 4°C.

Aspirate and discard the supernatant fluid without disturbing the cell pellet.
– Note that the pellet will be very small.

5 Gently re-suspend the cell pellet in 500 µL of cold thawing media (4°C) and mix gently with a pipet. (Gently aspirate the media up into the pipet and then dispensing back down in the vial, do this 2-3 times.)

6 Count the cells and assess viability using the Trypan Blue exclusion assay. Add 10 µL of cell suspension to 10 µL of Trypan Blue solution, 0.4% (Please refer to the Cryopreserved Kupffer Cell Preparation Worksheet on ***page 64***).

7 Measure the volume of the cell suspension and q.s. to a final volume that yields 0.5 x 10^6 cells/mL or to the desired density per internal protocol.

8 Plate 0.5 mL of cell suspension per well of a 24-well cell culture-treated plate.

9 Place the cells in a humidified 37°C/5% CO_2 incubator and swirl in a figure 8 pattern to evenly distribute the cell suspension. (Note that the cells will begin to clump together as the media temperature increases; this is normal.)

10 Dosing with a test article can begin after the Kupffer cells have been in culture for 24-48 hours even though the cells remain in suspension. Cells will begin to attach at approximately day 5, at which point the media (Opti**Thaw**) can be changed. Prior to this time, a majority of the cells will be in suspension and nutrients could be replenished by addition of warm media (Opti**Thaw**).

Appendix: Kupffer Cells

Cryopreserved Kupffer Cell Preparation Worksheet

This worksheet may be used to record information during the preparation of your Kupffer cell sample. Prepare additional copies of this sheet as needed.

Kupffer Cell Sample Identification

# Vials Thawed	
Sample ID (Lot Number)	1.0 mL

Date of Kupffer cell isolation:_____

Trypan Blue Cell Count Analysis

A trypan blue exclusion analysis should be performed (step 6 in the thawing protocol) following re-suspension of the initial cell pellet.

Cells counted		*% Viability [A/(A+B)] x 100*	*Dilution factor[1]*	*Hemacytometer factor[2]*	*Volume of sample[3]*	*Number of viable Kupffer cells[4]*	*Final cell Concentration[5]*
Live	*Dead*						
A	**B**		**C**	**D**	**E**		
				10,000			
				10,000			

*[1] The dilution factor will equal 2 if a 10 µL aliquot of the cell suspension was dispensed into 10 µL of Trypan Blue solution as stated in the Protocol for Thawing Kupffer Cells (**page 63**) for subsequent counting. If an alternate means of dilution was used, a different dilution factor should be calculated.*

[2] The hemacytometer factor will typically equal 10,000. For more information consult your hemacytometer manufacturer.

[3] Volume of the sample indicates the total volume of the cell suspension from which the counting aliquot was removed.

[4] The number of viable Kupffer cells may be calculated from the following equation:

$$\frac{A}{\text{quadrants}} \times C \times D \times E$$ *where "quadrants" equals the number of quadrants counted on the hemacytometer*

[5] The desired concentration should be determined based on the specific requirements of your experimental design.

Sample Dilution

Use the following table to calculate the final volume needed to reach the desired cell concentration.

# of viable Kupffer cells (determined above)	*Desired cell concentration for use (million cells/mL)*	*Final volume (mL)*	*Volume of media to add to reach desired concentration (mL)*
F	**G**	**H**	**I**

H = F/G *I = H - Volume of sample*

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

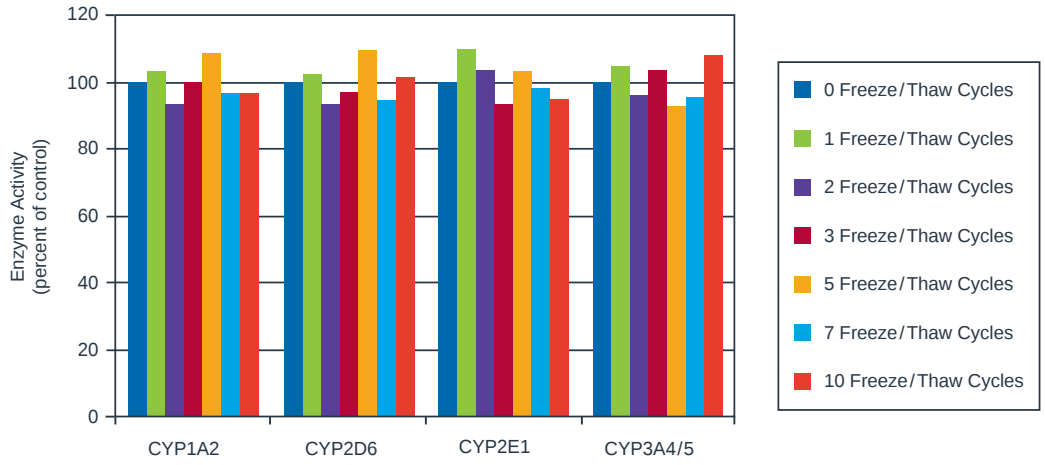
Comparison of XTreme 200 Pool vs. Pool of 50 Human Liver Microsomes

	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

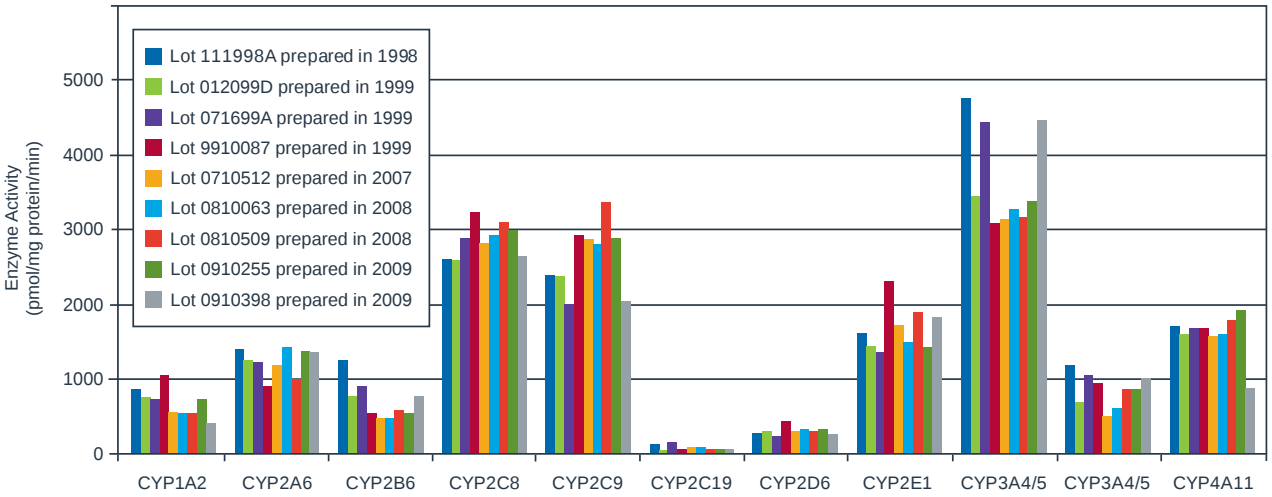
Human Liver Microsomes

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



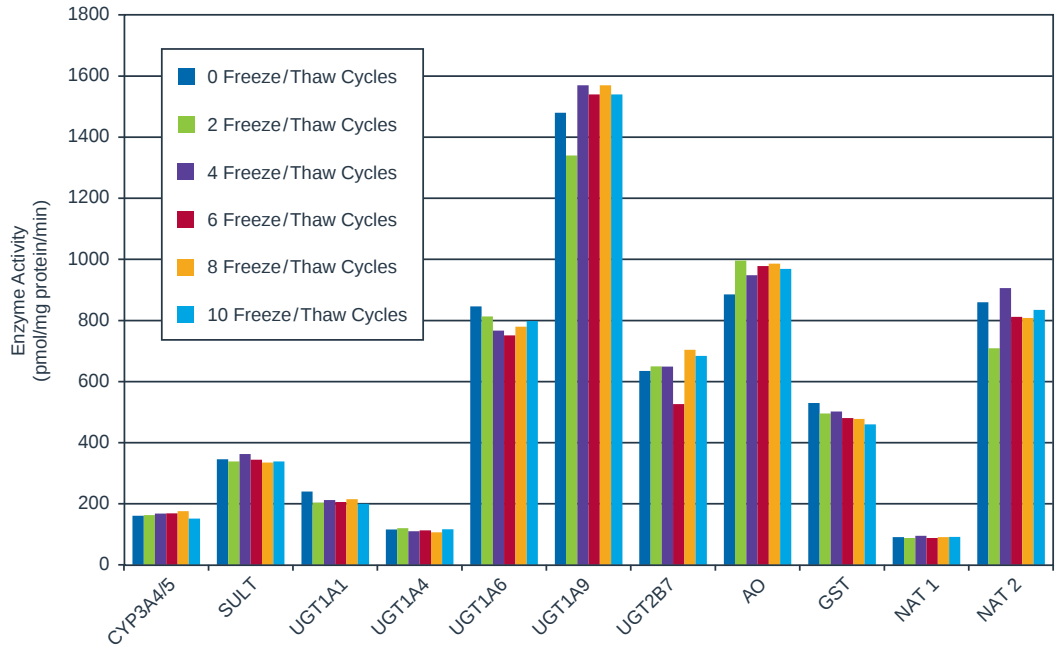
CYP450 Stability in Pooled Human Liver Microsomes

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



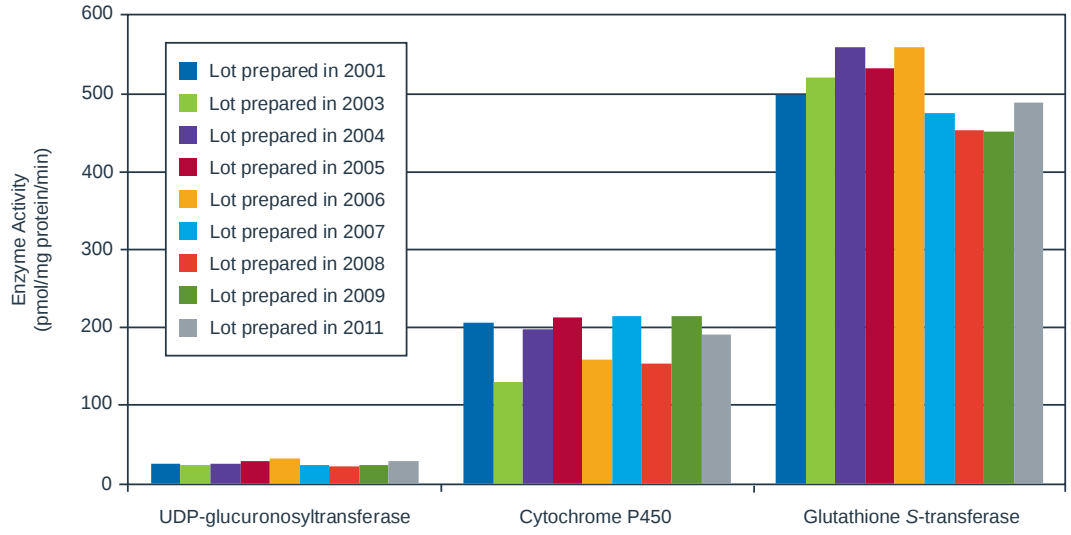
Human Liver S9 Pools

Effects of freeze / thaw cycles on enzyme activities in human mixed gender liver S9 pools



Enzyme Stability in Pooled Human Liver S9 Fractions

Stored at -70°C for up to 10 years
Samples analyzed in 2011 using the same assay conditions



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: 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, XenoTech's human liver microsomes are stable for >12 years.

Freezing and thawing samples

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: 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: Extrahepatic Subcellular Fractions

Lung, Kidney and Skin

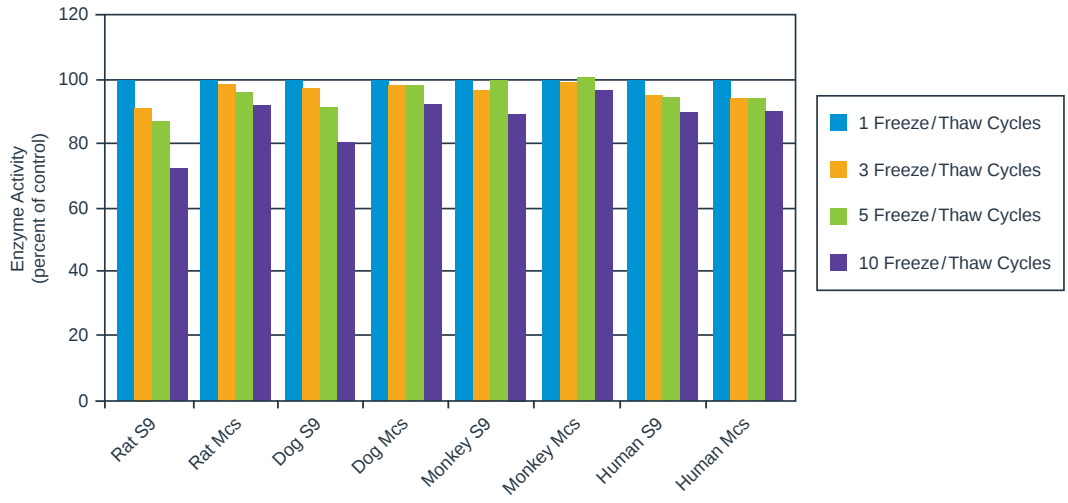
Preparation of Lung, Kidney and Skin Subcellular Fractions

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.

[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

Effect of repetitive freezing and thawing of pooled kidney S9 and microsomes on CYP4A (lauric acid 12-hydroxylase) activity



Appendix: Extrahepatic Subcellular Fractions

Intestine

Preparation of Intestine Subcellular Fractions

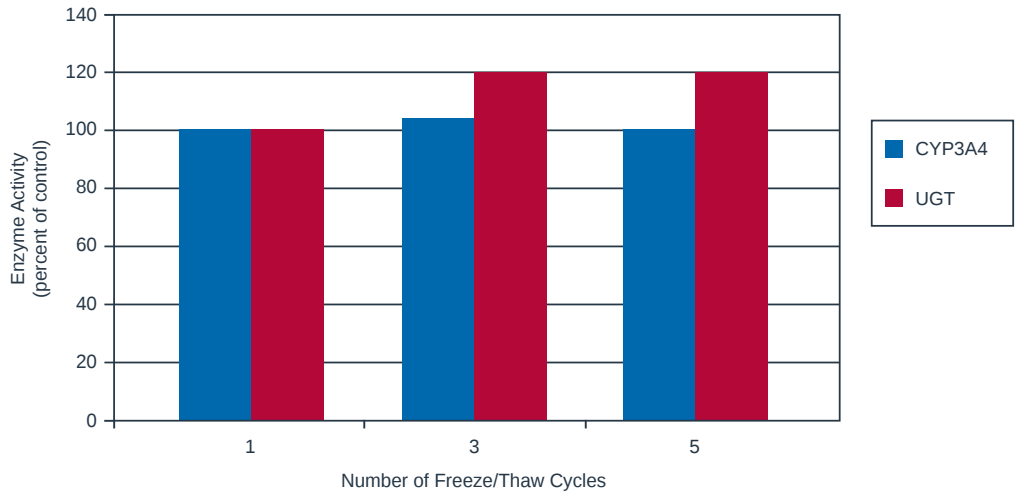
The intestinal lumen is rinsed with 0.9% saline solution or comparable solution to remove chyme or other partially digested material. Mature enterocytes are eluted from the intestinal lumen in the presence of an ethylenediamine-tetraacetic acid (EDTA)-based elution buffer containing protease inhibitors. The eluate is then subjected to multiple low-speed centrifugation steps to concentrate and wash the enterocytes. The concentrated enterocyte pellet is resuspended and homogenized in GI homogenization buffer[1]. Typically, 1 to 3 mL of GI homogenization buffer is added per gram of wet tissue weight to produce a 25-50% homogenate. The homogenate is centrifuged at 4,500-5,500 g_{max} for 5 ± 1 minutes at 0-8ºC, then at 11,500-12,500 g_{max} for 15 ± 1 minutes at 0-8ºC to prepare a post-mitochondrial supernatant (S9).

Microsomes are prepared by the ultracentrifugation of the post-mitochondrial supernatant (S9) fraction at 104,000-109,000 g_{max} for 60 ± 5 minutes at 0-8°C. This ultracentrifugation results in a cytosolic supernatant and a microsomal pellet. Microsomes are resuspended in 250 mM sucrose and stored at or below -70°C.

For XenoTech's PMSF-free products, the same method is used except we remove PMSF from the buffer solution.

[1]Homogenization Buffer
50 mM Tris-HCl, pH 7.4 at 4°C
150 mM Potassium chloride
1 mM EDTA, pH 7.4
20% Glycerol
Heparin
PMSF
Leupeptin
DTT
Aprotinin

Effect of repetitive freezing and thawing of pooled human intestine microsomes on CYP3A4 (testosterone 6β-hydroxylase) and UGT (4-methylumbelliferone glucuronidase) activities



Enzyme Activity Characterization Assays

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

Lung, kidney or intestine microsomes[1] (*e.g.*, 0.1 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 on the following page. 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 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 6β-hydroxytestosterone 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.

Internal Standards for Cytochrome P450 Characterization Assays

Substrate	*Internal Standard*
Phenacetin	Acetaminophen-d4
Ebastine	Hydroxyebastine-d5
Testosterone	6β-Hydroxytestosterone-d2
Lauric Acid	12-Hydroxylauric acid-d20

Appendix: Extrahepatic Subcellular Fractions

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

CYP	*Substrate*	*Substrate Concentration[1] (µM)*	*Substrate Solvent (final % v/v)*	*Metabolite Standards*
CYP1A2	Phenacetin	80	Methanol (1%)	1-200 ng/mL Acetaminophen
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
CYP4A11	Lauric Acid	100	Na_2CO_3/Methanol (102 µM/0.94%)	2-400 ng/mL 12-Hydroxylauric acid

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

Esterase Activity Characterization Assays

Carboxylesterases (CE) are microsomal glycoproteins and members of the α/β hydrolase family of enzymes that cleave carboxylic acid ester functional groups in xenobiotics. Human carboxylesterase 1 (hCE-1, CES1A1) preferentially catalyzes the hydrolysis of compounds esterified with a small alcohol group, and is widely distributed in many tissues but is present at very low levels in human intestinal microsomes. Human carboxylesterase 2 (hCE-2, CES2A1), preferentially hydrolyzes compounds with a relatively small acyl group and large alcohol group. It is present in high levels in microsomes from human intestine, liver and kidney.

The hydrolysis of clopidogrel to clopidogrel acid and 6α-methylprednisolone 21-hemisuccinnate to methylprednisolone are specific reactions used to measure the activity of hCE1 and hCE2, respectively.

Microsomes (*e.g.*, 0.05 mg/mL) are incubated at 37 ± 1°C in a 200-µL incubation mixture (final volume) containing potassium phosphate (50 mM, pH 7.4), $MgCl_2$ (3 mM) and EDTA (1 mM, pH 7.4).

The reactions are started by the addition of marker substrate at the final concentration indicated. The reactions may also be started by the addition of protein into buffer containing substrate. At a designated time (*e.g.*, 10 min), the reactions are stopped by the addition of stop reagent (2% formic acid in acetonitrile) containing internal standard (175 µL). Zero-time incubations serve as blanks. Blanks spiked with various concentrations of known metabolites serve as standards. If possible, a selected microsomal sample is also incubated for approximately half and twice the incubation period to verify that 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 LC/MS/MS.

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

Enzyme	*Substrate*	*Substrate Concentration (µM)*	*Internal Standard*	*Metabolite Standards*
hCE1	Clopidogrel	150	Clopidogrel acid-d4	0.05-10 µM Clopidogrel acid
hCE2	6α-Methylprednisolone 21-hemisuccinnate	750	6β-Hydroxytestosterone-d3	0.025-5 µM Methylprednisolone

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-methyl-umbelliferyl 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.

Extrahepatic microsomes and S9 (*e.g.*, 0.2 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). 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

Pooled Lung Subcellular Fraction Products

	Microsomes	***S9 Fraction***	***Cytosol***
Species Available	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse
Volume/Vial	0.5 mL	1.0 mL	1.0 mL
Characterization Provided			
Marker Substrate Reaction			
NADPH-cytochrome *c* reductase	✓	✓	
Phenacetin *O*-dealkylation	✓	✓	
4-Methylumbelliferone glucuronidation	✓	✓	

**All extrahepatic microsomes are supplied in 250 mM sucrose buffer.*
**Lung S9 and cytosolic fractions are supplied in 50 mM Tris-HCl, (pH 7.4 at 4°C), containing 150 mM KCl and 2 mM EDTA.*
**No characterization provided for cytosolic fractions.*

Appendix: Extrahepatic Subcellular Fractions

Pooled Kidney Subcellular Fraction Products

	Microsomes	*S9 Fraction*	*Cytosol*
Species Available	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse
Volume/Vial	0.5 mL	1.0 mL	1.0 mL
Characterization Provided			
Marker Substrate Reaction			
NADPH-cytochrome *c* reductase	✓	✓	
Lauric acid 12-hydroxylation	✓	✓	
4-Methylumbelliferone glucuronidation	✓	✓	

**All extrahepatic microsomes are supplied in 250 mM sucrose buffer.*
**Kidney S9 and cytosolic fractions are supplied in 50 mM Tris-HCl, (pH 7.4 at 4°C), containing 150 mM KCl and 2 mM EDTA.*
**No characterization provided for cytosolic fractions.*

Pooled Intestine Subcellular Fraction Products

	Microsomes	*S9 Fraction*	*Cytosol*
Species Available	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse
Volume/Vial	150 µL	1.0 mL	1.0 mL
Characterization Provided			
Marker Substrate Reaction			
NADPH-cytochrome *c* reductase	✓	✓	
Testosterone 6β-hydroxylation	✓	✓	
4-Methylumbelliferone glucuronidation	✓	✓	

**All extrahepatic microsomes are supplied in 250 mM sucrose buffer.*
**Intestine S9 and cytosolic fractions are supplied in 50 mM Tris-HCl, (pH 7.4 at 4°C), containing 150 mM KCl and 150 mM KCl and 1 mM EDTA, 20% glycerol, heparin, PMSF, leupeptin, DTT and aprotinin.*
**No characterization provided for cytosolic fractions.*

Pooled Intestinal PMSF-Free Subcellular Fraction Products

	Microsomes	*S9 Fraction*	*Cytosol*
Species Available	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse	Human Monkey Dog Rat Mouse
Volume/Vial	150 µL	1.0 mL	1.0 mL
Characterization Provided			
Marker Substrate Reaction			
Methylprednisolone 21-hemisuccinate hydrolysis	✓	✓	

**All extrahepatic microsomes are supplied in 250 mM sucrose buffer.*
**Intestine S9 and cytosolic fractions are supplied in 50 mM Tris-HCl, (pH 7.4 at 4°C), containing 150 mM KCl and 150 mM KCl and 1 mM EDTA, 20% glycerol, heparin, leupeptin, DTT and aprotinin.*
**No characterization provided for cytosolic fractions.*

Pooled Skin Subcellular Fraction Products

	Microsomes	*S9 Fraction*
Species Available	Rat Mouse	Rat Mouse
Volume/Vial	250 µL	1.0 mL
Characterization Provided		
Marker Substrate Reaction		
NADPH-cytochrome *c* reductase	✓	✓
Testosterone 6β-hydroxylation	✓	✓
4-Methylumbelliferone glucuronidation	✓	✓
Clopidogrel hydrolysis	✓	✓
Methylprednisolone 21-hemisuccinate hydrolysis	✓	✓

**All extrahepatic microsomes are supplied in 250 mM sucrose buffer.*
**Skin S9 fractions are supplied 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.

Appendix: RapidStart™ NADPH Regenerating System

Product K5000

Suggested Use

In a typical microsomal incubation, the recommended concentration of the NADPH regenerating system consists of 1 mM NADP(H), 5 mM glucose-6-phosphate and 1 U/mL of glucose-6-phosphate dehydrogenase. These concentrations can be achieved by adding 3.50 mL of high purity water to the frozen components in the RapidStart™ vial, vortexing, and then adding the activated RapidStart™ solution to the incubation mixture in a 1:10 (v/v) dilution to initiate metabolic reactions.

RapidStart™ is designed as a flexible prepackaged NADPH regenerating system to accommodate various experimental designs. Depending on the amount of high purity water added to the system, RapidStart™ generates NADPH with a concentration of ~4-80 mM.

RapidStart™ Dilution Concentration Table

	NADP (mM)		*G6P (mM)*		*G6PDH (U/mL)*			
High Purity Water Added (mL)	*As Prepared*	*Final Incubation 1:10 (v/v)*	*As Prepared*	*Final Incubation 1:10 (v/v)*	*As Prepared*	*Final Incubation 1:10 (v/v)*	*Total Volume (mL)*	*Expected NADPH Concentration, As Prepared (mM)*
0.00	100.00	10.00	500.00	50.000	100.00	10.000	1.50	~70-90
0.50	25.00	2.50	125.00	12.500	25.00	2.500	2.00	~17-22
1.00	20.00	2.00	100.00	10.000	20.00	2.000	2.50	~14-18
2.00	14.29	1.43	71.43	7.143	14.29	1.429	3.50	~10-13
2.50	12.50	1.25	62.50	6.250	12.50	1.250	4.00	~8.8-11.2
3.00	11.11	1.11	55.56	5.556	11.11	1.111	4.50	~7.8-10
3.50*	10.00	1.00	50.00	5.000	10.00	1.000	5.00	~7.0-9.0
4.00	9.09	0.91	45.45	4.545	9.09	0.909	5.50	~6.4-8.2
4.50	8.33	0.83	41.67	4.167	8.33	0.833	6.00	~5.8-7.5
5.00	7.69	0.77	38.46	3.846	7.69	0.769	6.50	~5.4-6.9
5.50	7.14	0.71	35.71	3.571	7.14	0.714	7.00	~5.0-6.4
6.00	6.67	0.67	33.33	3.333	6.67	0.667	7.50	~4.7-6.0
6.50	6.25	0.63	31.25	3.125	6.25	0.625	8.00	~4.4-5.6
7.00	5.88	0.59	29.41	2.941	5.88	0.588	8.50	~4.1-5.3
7.50	5.56	0.56	27.78	2.778	5.56	0.556	9.00	~3.9-5.0
8.00	5.26	0.53	26.32	2.632	5.26	0.526	9.50	~3.7-4.7
8.50	5.00	0.50	25.00	2.500	5.00	0.500	10.00	~3.5-4.5

**Denotes XenoTech's recommended concentration.*

NADPH Concentration Determination

Although NADP and NADPH both absorb at 260 nm, only NADPH absorbs strongly at 340 nm. Consequently, the reduction of NADP to NADPH is associated with an increase in absorbance at ~340 nm. This increase in absorbance at ~340 nm can be used to determine the concentration of NADPH.

To verify the concentration of NADPH regenerated, a spectrophotometer is used to determine absorbance at ~340 nm. Dispense 995 µL of high purity water into a sample cuvette and 1000 µL into a reference cuvette. Record baseline absorbance from 230 to 380 nm. Add 5 µL of prepared RapidStart™ to the sample cuvette and record the absorbance from 230 to 380 nm. The concentration of NADPH in an incubation can be determined using the following equation:

$$\frac{\text{Absorbance}}{\varepsilon} \times \frac{\text{Volume of NADPH added to incubation mixture (µL)}}{\text{Incubation volume (µL)}} \times \frac{\text{Total volume in sample curvette (µL)}}{\text{Volume of NADPH added to curvette (µL)}} = \text{Concentration in incubation (mM)}$$

Where ε = extinction coefficient of NADPH = 6.22 $mM^{-1}cm^{-1}$ (which indicates that a 1 mM solution of NADPH would have an absorbance value of 6.22 in a cuvette with a 1 cm light path).

Product K5100

Suggested Use

In a typical microsomal incubation, the recommended concentration of the NADPH regenerating system consists of 1 mM NADP(H), 5 mM glucose-6-phosphate and 1 U/mL of glucose-6-phosphate dehydrogenase. These concentrations can be achieved by adding 0.700 mL of high purity water to the frozen components in the RapidStart™ vial, vortexing, and then adding the activated RapidStart™ solution to the incubation mixture in a 1:10 (v/v) dilution to initiate metabolic reactions.

RapidStart™ is designed as a flexible prepackaged NADPH regenerating system to accommodate various experimental designs. Depending on the amount of high purity water added to the system, RapidStart™ generates NADPH with a concentration of ~4-80 mM

RapidStart™ Dilution Concentration Table

	NADP (mM)		***G6P (mM)***		***G6PDH (U/mL)***			
High Purity Water Added (mL)	*As Prepared*	*Final Incubation 1:10 (v/v)*	*As Prepared*	*Final Incubation 1:10 (v/v)*	*As Prepared*	*Final Incubation 1:10 (v/v)*	*Total Volume (mL)*	*Expected NADPH Concentration, As Prepared (mM)*
0.10	25.00	2.50	125.00	12.500	25.00	2.500	0.40	~17-22
0.20	20.00	2.00	100.00	10.000	20.00	2.000	0.50	~14-18
0.30	16.67	1.67	83.33	8.333	16.67	1.667	0.60	~10-13
0.40	14.29	1.43	71.43	7.143	14.29	1.429	0.70	~8.8-12.8
0.50	12.50	1.25	62.50	6.250	12.50	1.250	0.80	~10.0-11.2
0.60	11.11	1.11	55.56	5.556	11.11	1.111	0.90	~7.8-10
0.70*	10.00	1.00	50.00	5.000	10.00	1.000	1.00	~7.0-9.0
0.80	9.09	0.91	45.45	4.545	9.09	0.909	1.10	~6.4-8.2
0.90	8.33	0.83	41.67	4.167	8.33	0.833	1.20	~5.8-7.5
1.00	7.69	0.77	38.46	3.846	7.69	0.769	1.30	~5.4-6.9
1.10	7.14	0.71	35.71	3.571	7.14	0.714	1.40	~5.0-6.4
1.20	6.67	0.67	33.33	3.333	6.67	0.667	1.50	~4.7-6.0
1.30	6.25	0.63	31.25	3.125	6.25	0.625	1.60	~4.4-5.6
1.40	5.88	0.59	29.41	2.941	5.88	0.588	1.70	~4.1-5.3
1.50	5.56	0.56	27.78	2.778	5.56	0.556	1.80	~3.9-5.0
1.60	5.26	0.53	26.32	2.632	5.26	0.526	1.90	~3.7-4.7
1.70	5.00	0.50	25.00	2.500	5.00	0.500	2.00	~3.5-4.5

**Denotes XenoTech's recommended concentration.*

NADPH Concentration Determination

Although NADP and NADPH both absorb at 260 nm, only NADPH absorbs strongly at 340 nm. Consequently, the reduction of NADP to NADPH is associated with an increase in absorbance at ~340 nm. This increase in absorbance at ~340 nm can be used to determine the concentration of NADPH.

To verify the concentration of NADPH regenerated, a spectrophotometer is used to determine absorbance at ~340 nm. Dispense 995 µL of high purity water into a sample cuvette and 1000 µL into a reference cuvette. Record baseline absorbance from 230 to 380 nm. Add 5 µL of prepared RapidStart™ to the sample cuvette and record the absorbance from 230 to 380 nm. The concentration of NADPH in an incubation can be determined using the following equation:

$$\frac{\text{Absorbance}}{\varepsilon} \times \frac{\text{Volume of NADPH added to incubation mixture (µL)}}{\text{Incubation volume (µL)}} \times \frac{\text{Total volume in sample curvette (µL)}}{\text{Volume of NADPH added to curvette (µL)}} = \text{Concentration in incubation (mM)}$$

*Where **ε** = extinction coefficient of NADPH = 6.22 $mM^{-1}cm^{-1}$ (which indicates that a 1 mM solution of NADPH would have an absorbance value of 6.22 in a cuvette with a 1 cm light path).*

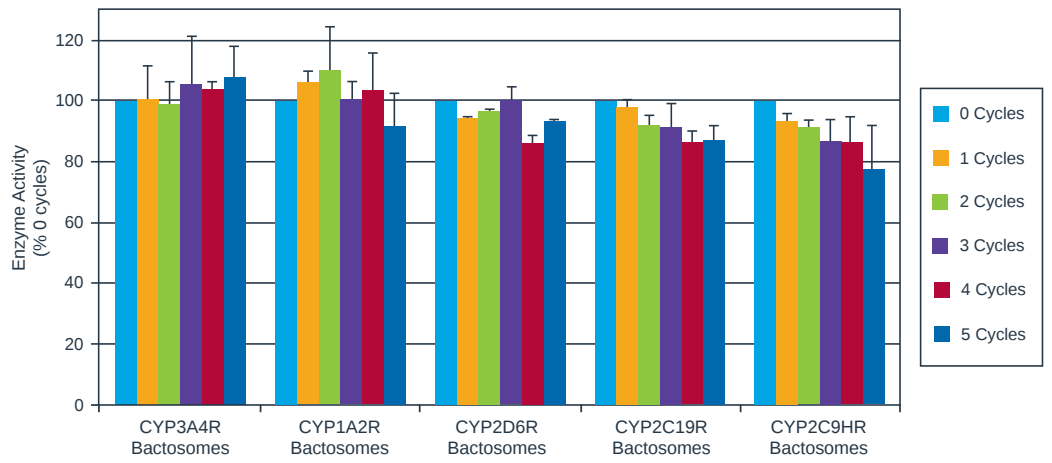
Appendix: Recombinant Enzymes

Classic and EasyCYP Bactosomes

What is the Difference Between R, HR and LR Bactosomes?

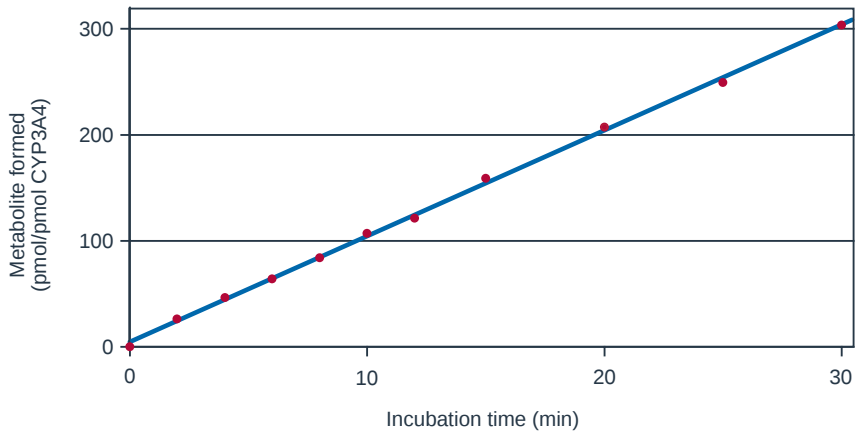
The activity of many cytochrome P450s can vary significantly with the amount of NADPH P450 reductase available. R and HR Bactosomes have high levels of NADPH P450 reductase and have, therefore, a high activity. This limits the linearity of substrate turnover with time. LR Bactosomes contain lower levels of NADPH P450 reductase than their R counterparts. These Bactosomes, while they have a lower V_{max} for a given substrate, show enhanced linearity of substrate turnover with time. For a good illustration of this compare the product sheets for CYP3A4R and CYP3A4LR. The product that you choose will depend on your assay requirements.

Freeze / Thaw Stability for Bactosomes



**Values +/- sd, n = 3 except * where n = 2 and values +/- range.*
**Bactosomes were thawed slowly on ice and refrozen rapidly on dry ice.*
**1 cycle = thawing and refreezing once.*

Linearity with Time for CYP3A4BLR Bactosomes



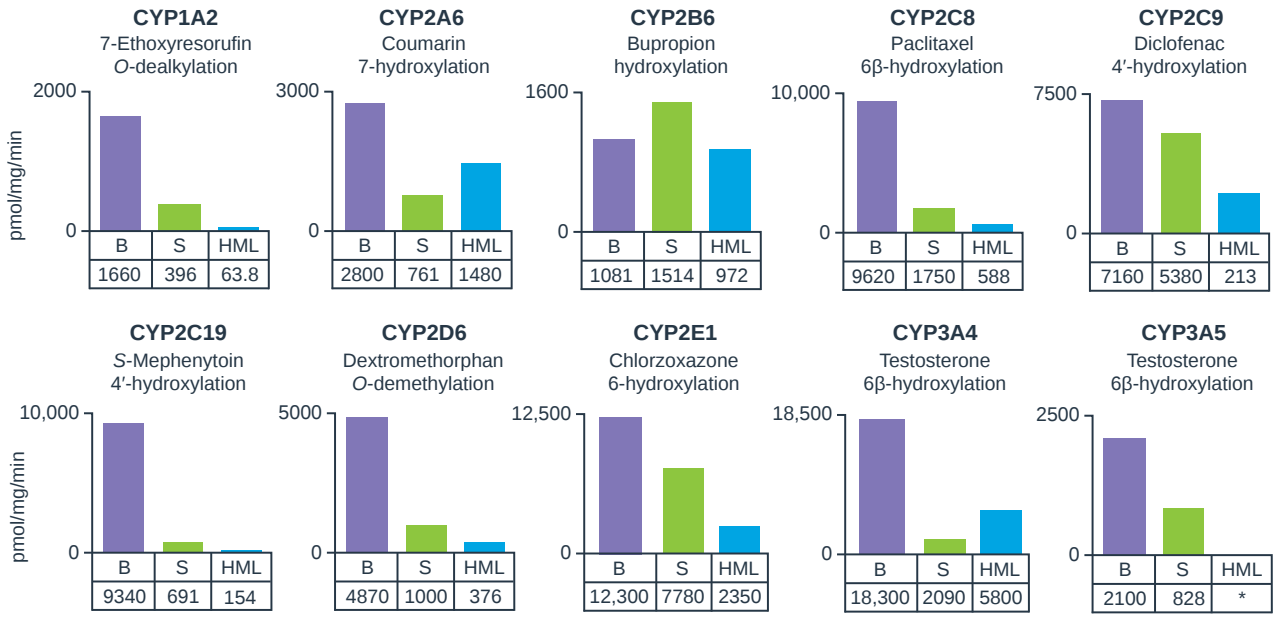
The graph above represents the concentration of 6β-hydroxytestosterone (pmol) formed per pmol of CYP3A4BLR over a 30 minute incubation.

Custom CYPs and special packaging requests available. Contact us at 913.438.7450 for more information.

Appendix: Recombinant Enzymes

Bactosomes vs. Supersomes

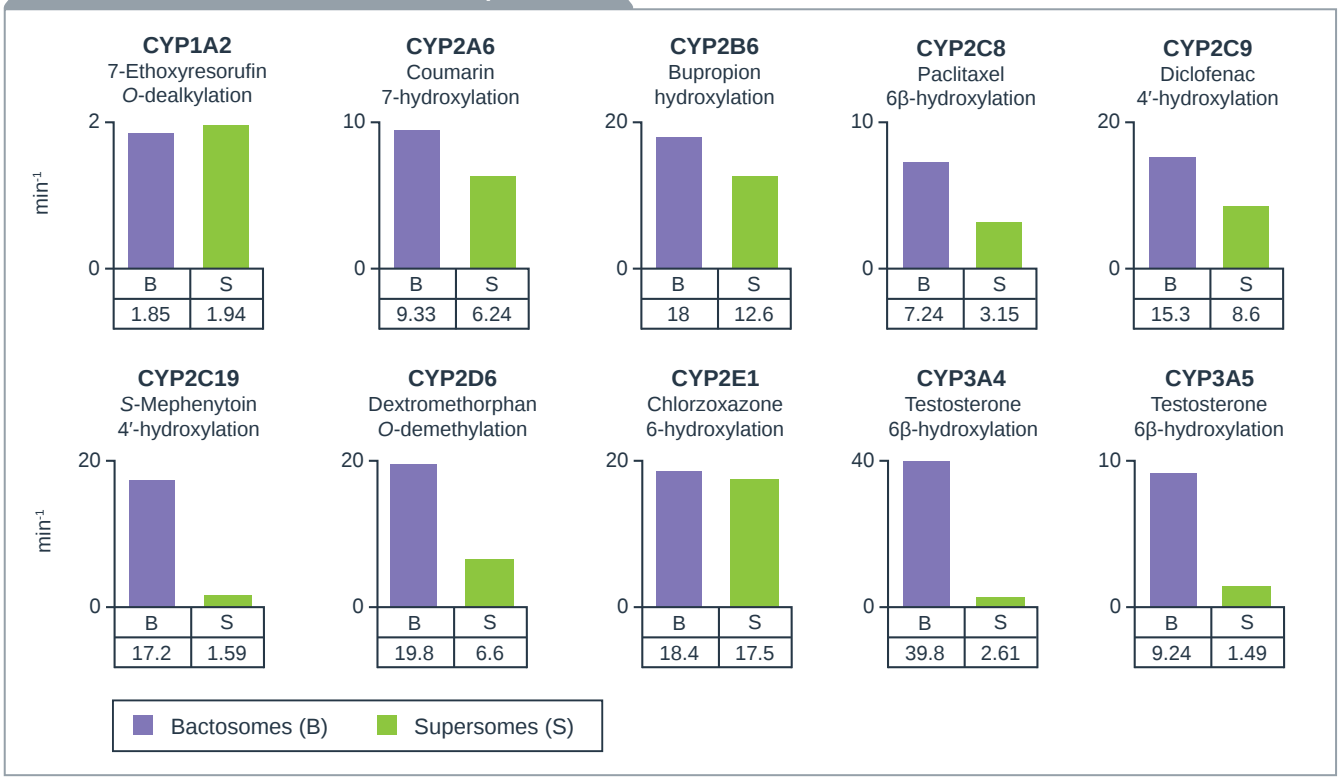
Enzymatic Rates: Bactosomes vs. Supersomes



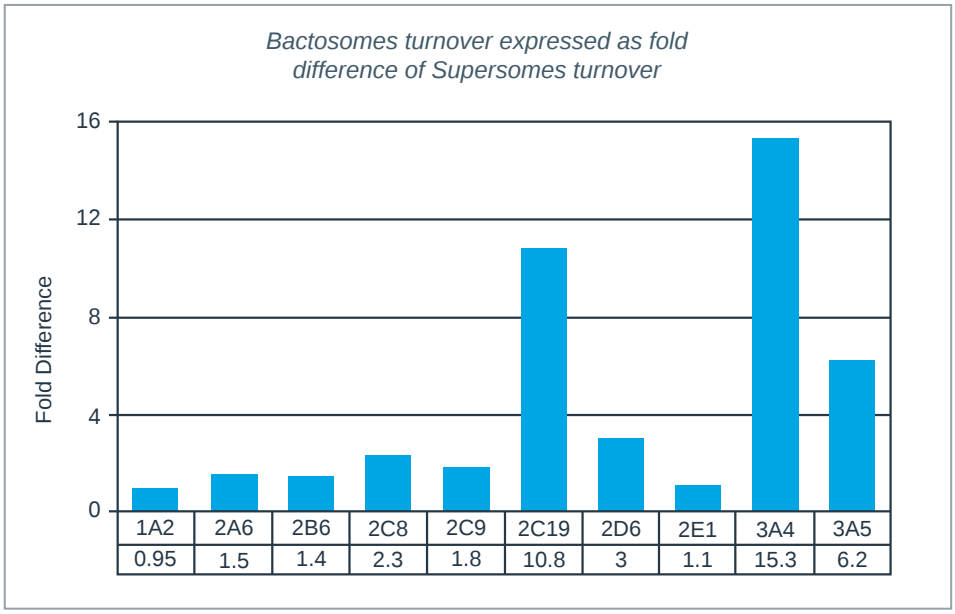
**In human liver microsomes, testosterone 6β-hydroxylation is catalyzed by both CYP3A4 and CYP3A5.*

Cypex Bactosomes also show excellent linearity over time, allowing longer incubations, generating better results.

Turnover Number: Bactosomes vs. Supersomes



Turnover number is mol motabolite formed per mol rCYP per min.



Appendix: Recombinant Enzymes

All assays are set up so that less than 15% (10% in most cases) of the substrate is used. The activity is determined from the amount of metabolite generated, NOT the amount of substrate used. Except where noted, all analysis is by HPLC with absorbance of fluorescence detection.

CYP	*Substrate*	*Metabolite*	*Buffer (final concentrations)*
CYP1A1	7-ethoxycoumarin	Umbelliferone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP1A1	7-ethoxyresorufin	Resorufin	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP1A2	7-ethoxycoumarin	Umbelliferone	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP1A2	7-ethoxyresorufin	Resorufin	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP1B1 (all allelic variants)	17β-estradiol	4-hydroxyestradiol	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP1B1 (all allelic variants)	7-ethoxyresorufin	Resorufin	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2A6 ± cytochrome b_5	Coumarin	Umbelliferone	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2A13	Coumarin	Umbelliferone	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2B6 ± cytochrome b_5	Diazepam	Desmethyldiazepam	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2B6 ± cytochrome b_5	7-ethoxy 4-trifluoromethylcoumarin	7-hydroxy 4-trifluoromethylcoumarin	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2B6 ± cytochrome b_5	Bupropion	Hydroxybupropion	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C8 ± cytochrome b_5	Paclitaxel	6α-hydroxypaclitaxel	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C9 ± cytochrome b_5	Diclofenac	4′-hydroxydiclofenac	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C9 ± cytochrome b_5	Tolbutamide	Hydroxytolbutamide	50 mM potassium phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C9*2 ± cytochrome b_5	Diclofenac	4′-hydroxydiclofenac	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C9*2 ± cytochrome b_5	Tolbutamide	Hydroxytolbutamide	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C9*3 ± cytochrome b_5	Diclofenac	4′-hydroxydiclofenac	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C9*3 ± cytochrome b_5	Tolbutamide	Hydroxytolbutamide	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C18	Diclofenac	4′-hydroxydiclofenac	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C19 ± cytochrome b_5	Diazepam	Desmethyldiazepam	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C19 ± cytochrome b_5	*S*-mephenytoin	4′-hydroxymephenytoin	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2C19	Tolbutamide	Hydroxytolbutamide	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2E1	Chlorzoxazone	6-hydroxychlorzoxazone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2E1 + cytochrome b_5	Chlorzoxazone	6-hydroxychlorzoxazone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2D6	Dextromethorphan	Dextrorphan	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2D6*2	Dextromethorphan	Dextrorphan	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2D6*10	Dextromethorphan	Dextrorphan	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2D6*39	Dextromethorphan	Dextrorphan	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2D6 (all alleles)	Bufuralol	1′-hydroxybufuralol	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP2J2	Terfenadine	Terfenadine alcohol	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP3A4 ± cytochrome b_5	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP3A5 ± cytochrome b_5	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP3A7	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP3A7 + cytochrome b_5	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP4A11 ± cytochrome b_5	Lauric acid	12-hydroxylauric acid	79 mM Tris-HCL pH 7.4, 5 mM Magnesium Chloride
CYP4F2 + cytochrome b_5	Leukotriene B4	20-hydroxyleukotriene B_4	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
CYP4F3B ± cytochrome b_5	Leukotriene B4	20-hydroxyleukotriene B_4	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride

⇦ *Table continues across from previous page.*

Substrate Concentration (time course)	CYP Concentration (time course) (pmol/mL)		CYP concentration (kinetics assay) (pmol/mL)		Substrate Concentration Range (kinetics assay)	Incubation time (kinetics assay)
	Low Reductase	High Reductase	Low Reductase	High Reductase		
5.0 µM	1.0	0.5	1.0	0.5	2 - 40 µM	5 min
0.3 µM	2.0	0.5	2.0	0.5	0.02 - 0.4 µM	15 sec
2.0 µM	10	2.5	12.5	2.5	1 - 16 µM	5 min
0.3 µM	5.0	2.0	7.5	5.0	0.05 - 1.0 µM	15 sec
4.0 µM	12.5		20		0.2 - 2.0 µM	5 min
0.3 µM	2.0		2.0		0.02 - 0.4 µM	1 min
0.8 µM		0.5		0.5	0.1 - 4.0 µM	5 min
0.2 µM	10	2.0	10	2.5	0.1 - 4.0 µM	5 min
50 µM (Low reductase 100 µM)	50	12.5	25	12.5	40 - 500 µM	5 min
2.0 µM	10 (+ cyt b_5 not tested)	2.5	12.5 (+ cyt b_5 not tested)	2.5	0.5 - 15 µM	5 min
40 µM (Low reductase 60 µM)	25 (+ cyt b_5 not tested)	7.5	25 (+ cyt b_5 not tested)	12.5	5 - 100 µM	5 min
4.0 µM	25	10	50	10	1.25 - 25 µM (low reductase 1 - 15 µM)	5 min
2.5 µM	7.5	3.0	10	2.0	1 - 20 µM	5 min
80 µM	75	15	50	10	40 - 500 µM	5 min
2.5 µM	7.5		10		1 - 20 µM	5 min
80 µM	75		50		40 - 500 µM	5 min
5.0 µM	7.5		10		2 - 20 µM	5 min
350 µM	100		50		40 - 500 µM	20 min
40 µM	250		150		20 - 200 µM	10 min
20 µM	50	25	50	50	4 - 50 µM (low reductase 10 - 100 µM)	5 min
30 µM	50	50	50	25	10 - 200 µM	5 min
125 µM	50	20	50	20	40 - 500 µM	5 min
120 µM		50		25	50 - 500 µM	5 min
40 µM (EasyCYP 120 µM)		50 (EasyCYP 25)		25	10 - 100 µM (EasyCYP 40 - 500 µM)	5 min
0.4 µM	5.0	1.0	10	1.0	0.1 - 2.0 µM	5 min
0.4 µM		1.0		1.0	0.2 - 4.0 µM	5 min
5.0 µM		5.0		2.0	2 - 40 µM	5 min
0.4 µM		1.0		1.0	0.2 - 4.0 µM	5 min
2 µM (CYP2D6*10 5 µM)	4.0	1.0 (CYP2D6*10 5)	5.0	1.25 (*2 1.0, *10 10)	0.5 - 20 µM	5 min
1.0 µM	0.125		0.0625		0.05 - 0.6 µM	5 min
32 µM	5.0	5.0	12.5	5.0	40 - 200 µM	5 min
60 µM	12.5	5.0	25	5.0	60 - 320 µM	5 min
32 µM	50	50	50	20	30 - 100 µM	5 min
32 µM	50	50	50	20	30 - 100 µM	5 min
10 µM (1:2 radio-labelled:non-labelled)	50 (+ cyt b_5 not tested)	10	50 (+ cyt b_5 not tested)	6	1 - 10 µM	5 min
20 µM	7.5		7.5		10 - 125 µM	5 min
10 µM	25	2.0	25	3.0	1 - 20 µM (low reductase 0.5 - 10 µM)	5 min

**All concentrations are final concentrations in the assay after the addition of cofactor.*
**NADPH generating system is used for the CYP assays (5 mM NADP, 25 mM glucose-6-phosphate, 5 U/mL glucose-6-phosphate dehydrogenase in 50 mM potassium phosphate pH 7.4).*

Appendix: Recombinant Enzymes

CYP	Substrate	Metabolite	Buffer (final concentrations)
Dog CYP1A1	7-ethoxycoumarin	Umbelliferone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP1A2	7-ethoxycoumarin	Umbelliferone	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP2B11	Diazepam	Desmethyldiazepam	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP2B11	Bupropion	Hydroxybupropion	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP2C21	Diclofenac	4′-hydroxydiclofenac	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP2C41	Diclofenac	4′-hydroxydiclofenac	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP2D15	Bufuralol	1′-hydroxybufuralol	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP3A12	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Dog CYP3A26	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Mouse CYP2B10	Bupropion	Hydroxybupropion	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Mouse CYP3A11	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Rat CYP2D4	Dextromethorphan	Dextrorphan	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Rat CYP2D18	Dextromethorphan	Dextrorphan	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Rat CYP3A9	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Cynomolgus CYP2C8 (CYP2C20)	Paclitaxel	6α-hydroxypaclitaxel	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Cynomolgus CYP3A4	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Cynomolgus CYP3A5	Testosterone	6β-hydroxytestosterone	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Cynomolgus CYP2C43	Testosterone	4-androstene-3,17-dione	100 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Cynomolgus CYP2C75	Tolbutamide	Hydroxytolbutamide	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
Cynomolgus CYP2C76	Tolbutamide	Hydroxytolbutamide	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride

Non-CYP Enzyme	Substrate	Metabolite	Buffer (final concentrations)
SULT1A1*1	*p*-nitrophenol	*p*-nitrophenylsulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1A1*1	4-methylumbelliferone	4-methylumbelliferone sulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1A1*2	4-methylumbelliferone	4-methylumbelliferone sulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1A2	1-naphthol	1-naphthylsulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1A3	1-naphthol	1-naphthylsulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1B1	4-methylumbelliferone	4-methylumbelliferone sulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1E1	[3H]17β-estradiol	17β-estradiol 3-sulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1C2	*p*-nitrophenol	*p*-nitrophenylsulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1C4	4-methylumbelliferone	4-methylumbelliferone sulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT2A1	17β-estradiol	17β-estradiol 3-sulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1C2	*p*-nitrophenol	*p*-nitrophenylsulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
SULT1C4	4-methylumbelliferone	4-methylumbelliferone sulfate	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
UGT1A6	4-methylumbelliferone	4-methylumbelliferone glucuronide	50 mM Potassium Phosphate pH 7.4, 5 mM Magnesium Chloride
UGT1A6	1-naphthol	1-naphthol glucuronide	50 mM Potassium Phosphate pH 7.4, 10 mM Magnesium Chloride
GSTA1, GSTM1†	1-chloro-2,4-dinitrobenzene		100 mM Potassium Phosphate pH 7.4
Aldehyde Oxidase	Phthalazine	1-phthalazinone	25 mM Potassium Phosphate pH 7.4, 0.1 mM EDTA
ALDH1A1	4-nitrobenzaldehyde	4-nitrobenzoic acid	50 mM HEPES pH7.4, 1 mM EDTA
CES1††	4-nitrophenylbutyrate	4-nitrophenol	50 mM Potassium Phosphate pH 7.4
CES2††	4-nitrophenylbutyrate	4-nitrophenol	50 mM Potassium Phosphate pH 7.4

†This assay is run at 37°C in a spectrophotometer, the OD_{340nm} is monitored over time.
††This assay is run at 30°C in a spectrophotometer, the OD_{340nm} is monitored over time.

⇦ *Table continues across from previous page.*

Substrate Concentration (time course)	CYP Concentration (time course) (pmol/mL)		CYP concentration (kinetics assay) (pmol/mL)		Substrate Concentration Range (kinetics assay)	Incubation time (kinetics assay)
	Low Reductase	High Reductase	Low Reductase	High Reductase		
5.0 µM	1.0		1.0		2 - 40 µM	5 min
1.0 µM	2.5		2.5		0.5 - 10 µM	5 min
5.0 µM	2.5		2.5		1 - 50 µM	5 min
25 µM	25		25		5 - 100 µM	5 min
30 µM	100		100		10 - 200 µM	5 min
500 µM	150		150		25 - 500 µM	40 min
3.0 µM	5.0		5.0		1 - 40 µM	5 min
32 µM	7.5		25		40 - 200 µM	5 min
32 µM	50		50		40 - 200 µM	5 min
15 µM	6.25		25		5 - 100 µM	5 min
32 µM	10		12.5		10 - 200 µM	5 min
15 µM	20		10		5 - 50 µM	5 min
15 µM	10		10		5 - 50 µM	5 min
32 µM	50		50		40 - 200 µM	5 min
15 µM	125		125		5 - 50 µM	10 min
15 µM	25		20		10 - 60 µM	5 min
60 µM	7.5		25		40 - 120 µM	5 min
15 µM	10		10		5 - 160 µM	5 min
350 µM	100		50		40 - 500 µM	20 min
350 µM	125		100		100 - 500 µM	20 min

**All concentrations are final concentrations in the assay after the addition of cofactor.*
**NADPH generating system is used for the CYP assays (5 mM NADP, 25 mM glucose-6-phosphate, 5 U/mL glucose-6-phosphate dehydrogenase in 50 mM potassium phosphate pH 7.4).*

⇦ *Table continues across from previous page.*

Substrate Concentration (time course)	Protein Concentration (time course) (µg/mL)	Protein concentration (kinetics assay) (µg/mL)	Substrate Concentration Range (kinetics assay)	Incubation time (kinetics assay)
4.0 µM	2.5			
2.0 µM	2.5	5.0	0.2 - 2.0 µM	5 min
2.0 µM	5.0	5.0	1.5 - 8.0 µM	5 min
1.2 µM	2.5	2.5	0.1 - 1.0 µM	5 min
5.0 µM	2.0	0.5	1 - 12 µM	5 min
20 µM	5.0	20	5 - 50 µM	5 min
0.02 µM	0.01**	0.01**	2 - 20 µM	5 min
250 µM	125	50	3000 - 9000 µM	5 min
15 µM	1.0	1.0	5 - 50 µM	5 min
1.0 µM	12.5	7.5	0.5 - 10 µM	5 min
250 µM	125	50	3000 - 9000 µM	5 min
15 µM	1.0	1.0	5 - 50 µM	5 min
200 µM	80	100	50 - 1000 µM	5 min
40 µM	100	100	10 - 400 µM	5 min
		0.2	250 - 3000 µM	2 min♦
5.0 µM	625	500	1 - 40 µM	2 min
2.0 µM	0.1	0.1	1 - 10 µM	4 min
		15	30 - 750 µM	20 min♦♦
		5.0	30 - 750 µM	20 min♦♦

***Supplemented to 1 µg/mL (time course) and 0.05 µg/mL (kinetics) with control cytosol.*
♦Continuously monitored in spectrophotometer with control protein in blank.
♦♦Continuously monitored in plate reader with 0 µg/mL protein in blank

Notes

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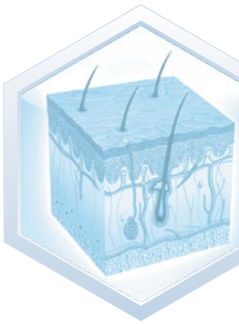

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ADRENAL GLAND | BRAIN | COLON | DUODENUM | EYE | HEART | ILEUM | JEJUNUM | KIDNEY | LIVER | LUNG | OVARY | SKIN | SPLEEN | STOMACH | TESTI

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