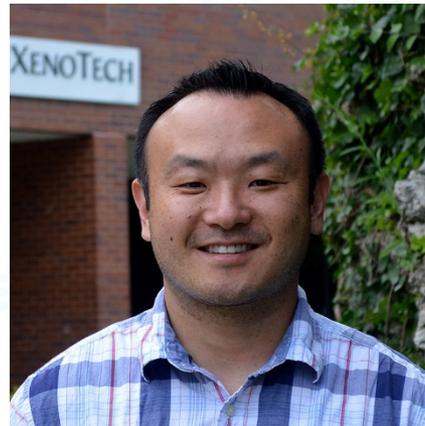


PROVEN GLOBAL CONTRACT RESEARCH EXPERTISE
FROM DISCOVERY THROUGH CLINICAL SUPPORT

What are Subcellular Fractions and Which Ones Should I Use?



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Global Technical Support - Products

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What are Subcellular fractions?

They are a non-living, in vitro matrix prepared from tissue homogenates/cellular lysates that have undergone various centrifugation steps to either enrich for or diminish specific enzymatic activities found in the original organs/tissue(s).

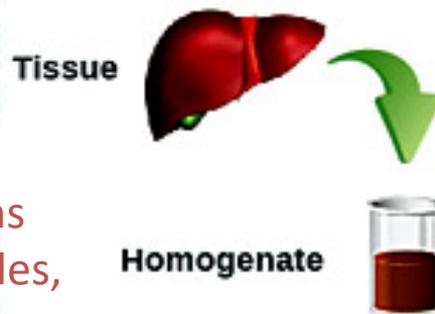
Vast majority of these are pooled from many different donors, however there are instances where subcellular fractions from individual donors are more advantageous.

How is it Made?

Tissue



How is it Made?

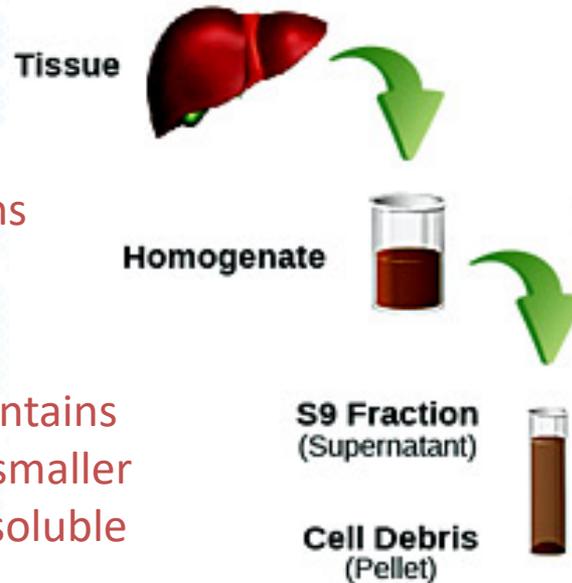


Homogenate contains membranes, organelles, and soluble proteins.

Mechanically homogenize the intact tissue

Clarified by low speed centrifugation to remove unbroken cells, connective tissue, and nuclei.

How is it Made?

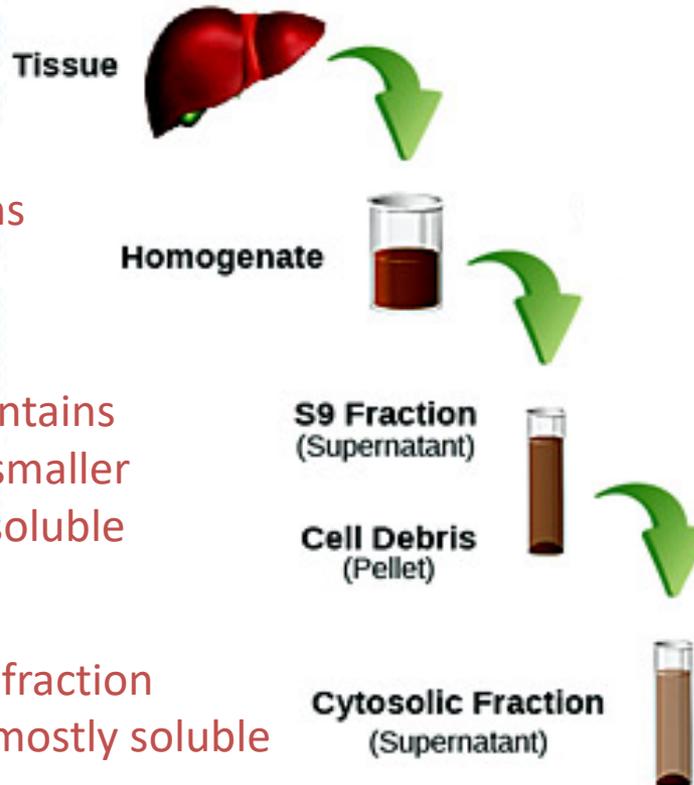


Homogenate contains PM, organelles, and soluble proteins.

S9 fraction contains microsomes, smaller vesicles, and soluble proteins

Homogenate is centrifuged at medium speed to pellet cell debris and larger organelles/vesicles (lysosomes/ Endosomes/mitochondria/ large membrane fragments).

How is it Made?



Homogenate contains PM, organelles, and soluble proteins.

S9 fraction contains microsomes, smaller vesicles, and soluble proteins

Cytosolic fraction contains mostly soluble proteins

S9 fraction is centrifuged at high speed to pellet microsomes and small vesicles/membrane fragments).

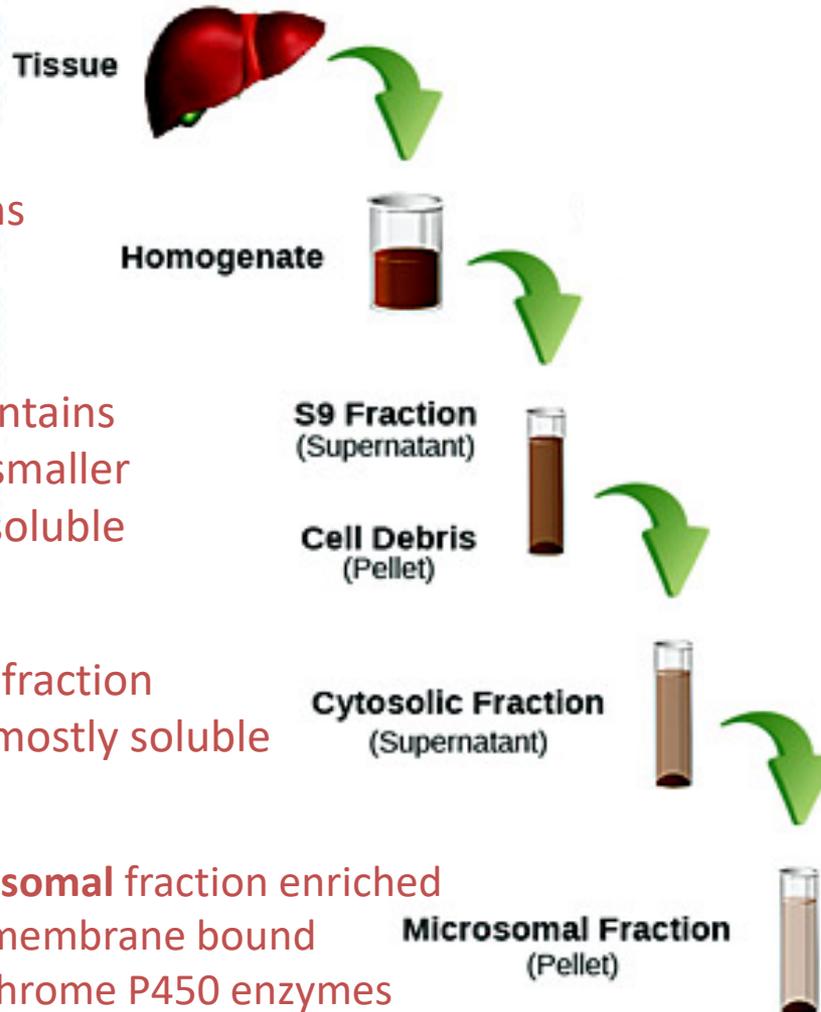
How is it Made?

Homogenate contains PM, organelles, and soluble proteins.

S9 fraction contains microsomes, smaller vesicles, and soluble proteins

Cytosolic fraction contains mostly soluble proteins

Microsomal fraction enriched with membrane bound Cytochrome P450 enzymes



Microsomal pellet is washed and spun again at high speed to repellet and further enrich the microsomes

DMPK/ADME pre-clinical studies

- In vitro DMPK studies to understand/predict in vivo ADME

- XenoTech products are useful in these experiments

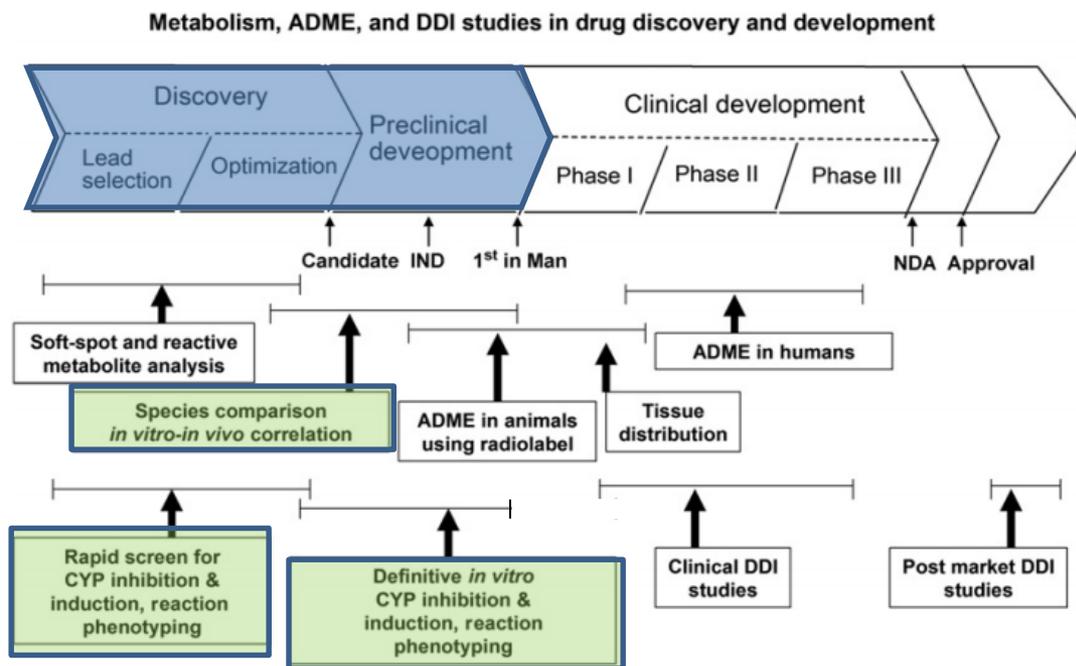


Figure 1 Typical model systems used in staged drug discovery and development.

- Drug Metabolism Pharmacokinetics
- Absorption, Distribution, Metabolism, Elimination

What Are They Used For?

- Metabolic Stability
- Reaction Phenotyping
- Metabolite Characterization
- CYP Inhibition
- Species Comparisons (selection of small animal models)
- Gender/Demographic Specific Differences
- Genetic Polymorphism
- Toxicity Prediction
- DDI Prediction
- IVIVE Modeling

Advantages of Subcellular Fractions

- Cheaper and easier to use than primary Hepatocytes
- Large donor pools minimize lot-to-lot variation
- Long-term lot availability
- Microsomes and S9 stable for 12+ years (unpublished data suggests significantly longer, stay tuned!)
- Matching S9 and microsomal donor pools
- Liver, intestine, lung, kidney, skin
- Robust preparation procedures
- Common small animal models
- Unsurpassed quality control

Product Options

- Genotyped
- High AO/XO activity
- NASH/ASH
- Gender-Specific
- Pool of 50
- XTreme 200

Human Liver Subcellular Fractions

	Pool of 200	Pool of 50
CYP Activity	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 Km for all major CYPs, UGTs and FMO. Also includes kinetic constants for major CYPs	Includes enzymatic rates at multiple times Km 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

Animal Liver Subcellular Fractions

- **Monkey**
 - Male* and Female* Cynomolgus
 - Male and Female Rhesus
 - **Minipig**
 - Male Gottingen
 - Male Yucatan
 - Male Sinclair
 - **Dog**
 - Male* and Female Beagle
 - **Rabbit**
 - Male and Female New Zealand White
 - **Guinea Pig**
 - Male Hartley Albino
 - **Hamster**
 - Male Golden Syrian
 - **Rat**
 - Male* and Female IGS Sprague-Dawley
 - Male and Female Fischer 344
 - Male and Female Wistar
 - Male Wistar Han
 - **Mouse**
 - Male and Female CD-1
 - Male B6C3F1
 - Male Balb/c
 - Male C57BL/6
- * Hepatic subcellular fractions from treated animals to induce specific P450 activities.

Extrahepatic Subcellular Fractions

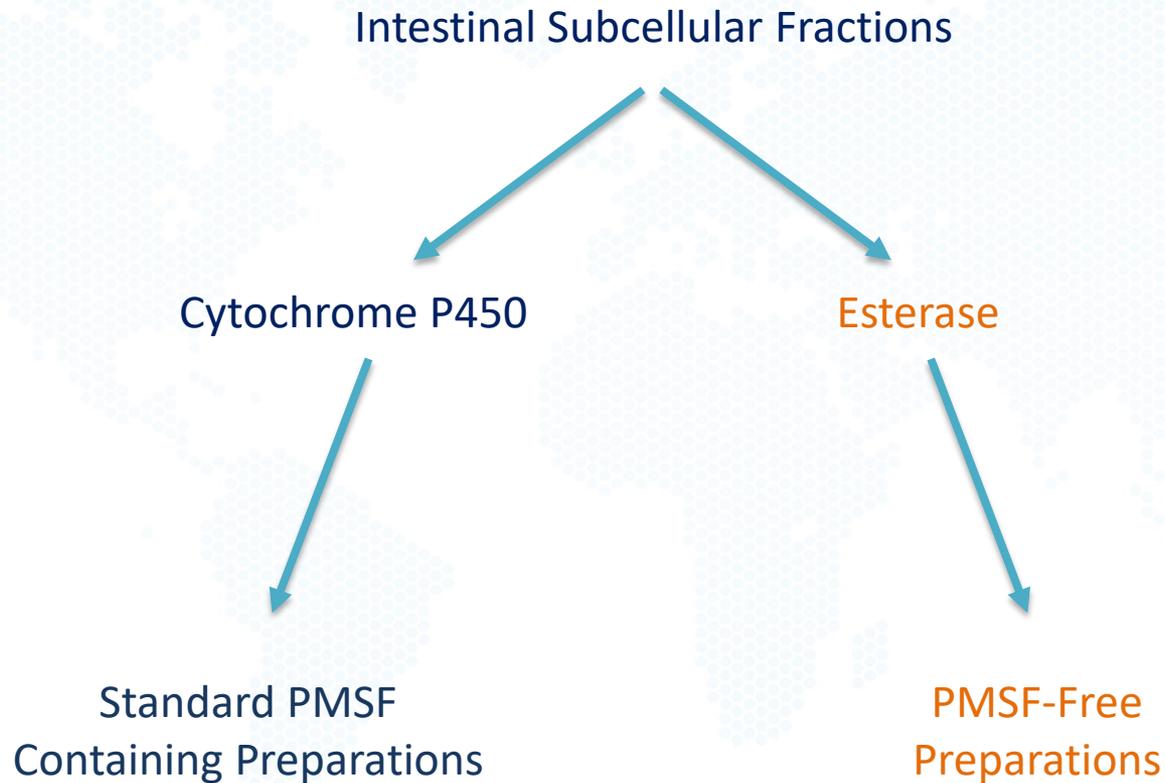
- **Human**
 - Mixed-gender intestine
 - Mixed-gender lung (non-smoker)
 - Mixed-gender lung (smoker)
 - Mixed-gender kidney
 - Mixed-gender skin
- **Cynomolgus Monkey**
 - Male intestine
 - Male lung
 - Male kidney
- **Beagle Dog**
 - Male intestine
 - Male lung
 - Male kidney
- **IGS Sprague-Dawley Rat**
 - Male intestine
 - Male lung
 - Male kidney
 - Male skin
- **CD-1 Mouse**
 - Male intestine
 - Male lung
 - Male kidney
 - Male skin
- **Gottingen Minipig**
 - Mixed-gender skin

Intestinal Subcellular Fractions

- Protease inhibitor cocktail used in standard intestinal preparations
 - Required because of unique, protease-rich environment of the intestinal lumen
 - protect and preserve the metabolic enzymatic activities of the subcellular fractions
 - Includes Heparin, Leupeptin, DTT, Aprotinin, and **PMSF**
- **PMSF = decreased esterase activities**
 - Standard PMSF fractions maintain high P450 (and other enzyme) activities
- **PMSF Free = high endogenous esterase activities**
 - Decreased metabolic enzymatic activities (extent of the decrease varies between species)

*No other subcell fractions are produced in the presence of PMSF because they are not exposed to the high levels of endogenous proteases inherently found within the gut and do not require intervention with protease inhibitors.

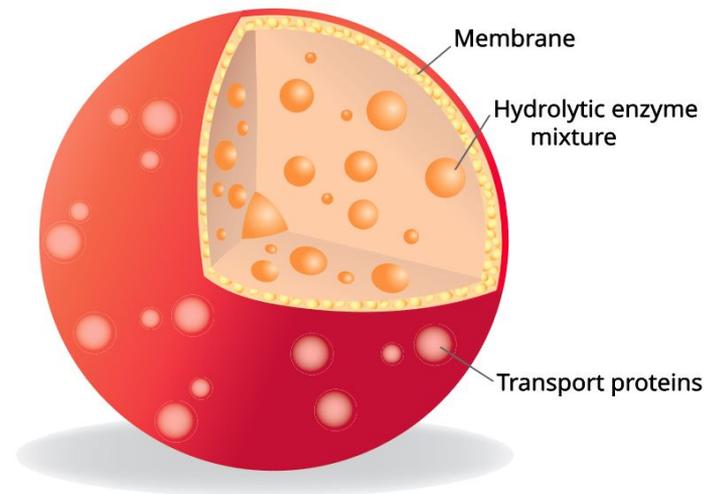
Standard or PMSF-free?



Non-Traditional Subcellular Fractions

Tritosomes/Lysosomes

LYSOSOME



What are Tritosomes and Lysosomes used for?

Biologic Stability/Metabolism/Safety

- ADCs
- Oligo/RNAi
- Recombinant proteins/peptides
- Nano Particles
- Antibiotics

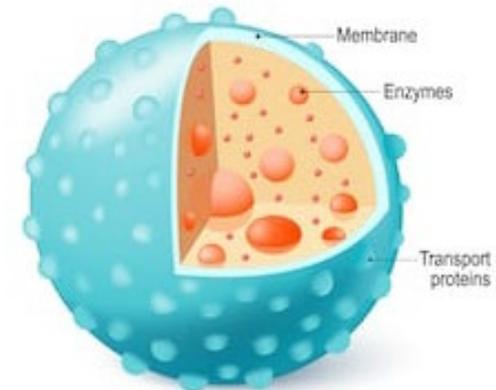
Rat Liver Tritosomes

Highly purified lysosomes, based on density purification of triton loaded rat liver lysosomes.

High specific activity of lysosomal enzymes and activities

- Acid phosphatase
- Cathepsin B
- RNase

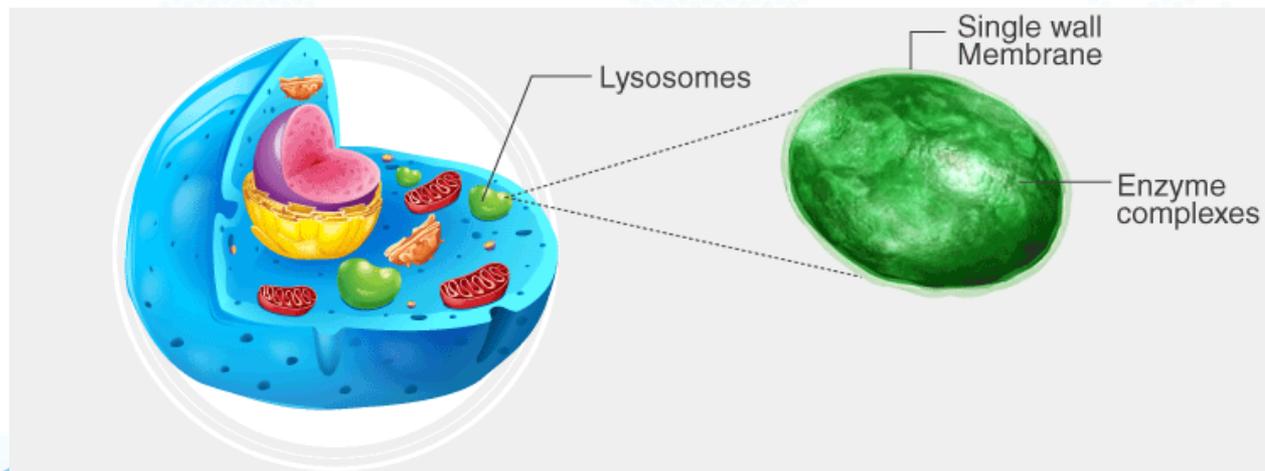
Simplified *in vitro* system for lysosomal targeted/mediated metabolism studies



<https://www.shutterstock.com/image-vector/anatomy-lysosome-hydrolytic-enzymes-membrane-transport-1167927220>

Human Liver Lysosomes

- Native human liver lysosomes are highly purified, have high specific activity.
- Characterized for the same enzymatic activities as Tritosomes.
- Lysosomes can catabolize proteins more efficiently than acidified S9

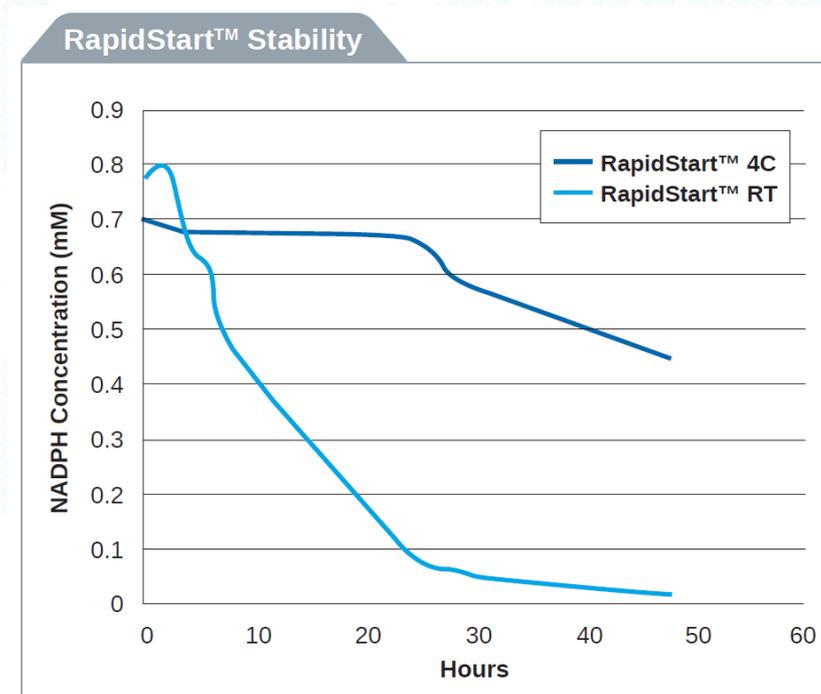


<https://byjus.com/biology/lysosomes/>

Should I use Homogenate, S9, Microsomes, Cytosol, and/or Lysosomes

- **Homogenate** represents the most complete entire proteasome of the tissue of interest (Phase I, II, and III activities). Generally lowest specific enzymatic activity.
- **S9** represents the majority of the tissue proteome, with decreases in enzymatic activities associated with larger organelles (mitochondria, endosome, lysosomes, large membrane vesicles). Higher specific activity than homogenate and are good for looking at phase I, II, III enzymatic activities in parallel.
- **Microsomes** are highly enriched for membrane bound Phase I, II, and III enzymatic activities and greatly reduced in activities of soluble cytosolic enzymes. Very high Phase I specific activities and UGT activities.
- **Cytosol** contains most of the Phase II enzymatic activities and soluble Phase I enzymes, but are depleted of microsomal enzymatic activities.
- **Lysosomes** contain organelle specific peptidases, nucleases, glucosidases, lipases, etc.

NADPH regenerating system or 1mM NADPH



Note: NADPH concentration measured over time at 4°C (4C) and at room temperature (RT)

GET-P450

**160+
Subcellular
Fraction
Products**

- 18 Species and strains
- 5 Major tissues: Liver, Intestine, Lung, Kidney, Skin
- Matching microsomes, S9 and cytosol
- Genotyped Individual HLM
- Treated monkey, dog and rat liver microsomes
- Custom Products surplus

Custom Products

- >20 years experience in custom-prepared products
- Subcellular fractions from various species and tissues
- Hepatocytes from highly-specific rodent strains
- Custom designed pools of human liver microsomes or custom aliquoting
- Use SXT proven methods or customer-provided methods
- Customer-driven protocols
- No minimum fee, but larger lots allows for volume efficiencies and lower price per mg
- Average time required to complete preparation: 2-12 weeks



Surplus Custom Products

Human and Animal Tissue Procurement

- Work with Organ Procurement Organizations for donated human tissue.
 - Non-transplantable tissue
 - Consent for research
 - Only obtain tissue from USA
- XenoTech has a strict set of criteria to ensure that livers are healthy and of the highest quality available.
- Negative serologies for major human pathogens, now including SARS-CoV-2.
- 24 hour on call team that can process the tissue the moment it arrives at our facility.
- All animals are procured through accredited vendors and are also free of major pathogens.
 - Animals are not housed on site.
 - Protocols are reviewed and IACUC approved
 - Have CITES for non-human primates



Thank You!

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- Drug Metabolism
- Enzyme Inhibition & Induction
- Protein Binding
- Metabolite Identification
- ADME Screening
- Toxicology

In Vivo ADME/PK & Distribution

- QWBA
- Microautoradiography
- Excretion / Mass Balance
- Tissue Distribution
- Blood / Plasma & Lymphatic Partition Rate

Bioanalytical

Pharmacology

- In Vitro Ligand Binding & Radioreceptor Assays
- Immunoassays

Chemical Synthesis

- Radiolabeled Synthesis
- Metabolite Synthesis
- Peptide Synthesis

Consulting...

Cellular Products

- Hepatocytes (Cryo/Fresh, Genotyped...)
- Non-Parenchymal Cells (Kupffer Cells)

Subcellular Fractions

- Liver Microsomes
- S9 Fractions
- Cytosol
- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
- Extrahepatic Fractions

Custom Products

- Various Species, Tissues & Preparations

Research Biobank

- Normal & Diseased Tissue Samples

Recombinant Enzymes

Substrates & Metabolites

Metabolite Production Kits

JCRB Cell Lines...