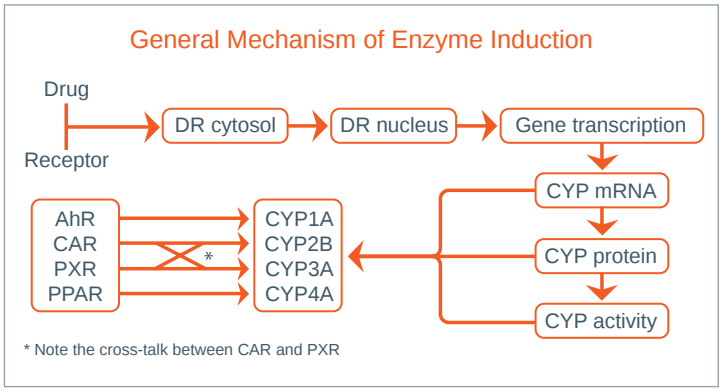
SEKISUI XENOTECH

XenoTech offers in vitro and ex vivo enzyme induction studies using FDA preferred substrates.

Enzyme induction studies assess the potential for a drug candidate to induce (up-regulate) the expression of drug-metabolizing enzymes. Enzyme induction can increase the metabolic clearance of a drug, thus compromising the drug's efficacy. Other adverse effects of induction include increased formation of an active metabolite, liver and/or thyroid tumor formation and toxicological consequences. The potential for enzyme induction to cause adverse effects has been recognized by the FDA and other regulatory agencies, who consider this type of study as an important part of *in vitro* drug development. Induction studies fall into two categories: *in vitro* and *ex vivo*.



In Vitro Enzyme Induction

In vitro studies analyze the test article for its perpetrator potential using primary cultures of hepatocytes from human and/or other species. By implementing the strictest selection criteria for suitable cultures; XenoTech ensures the use of the most robust *in vitro* test system for your induction study to deliver reliable and comprehensive results.

These studies provide information on clinically significant issues, such as:

- Pharmacokinetic tolerance
- Drug-drug interactions
- Enhanced biotransformation
- Enhanced bioactivation
- Increased elimination
- Explanation of *in vivo* animal tumor formation

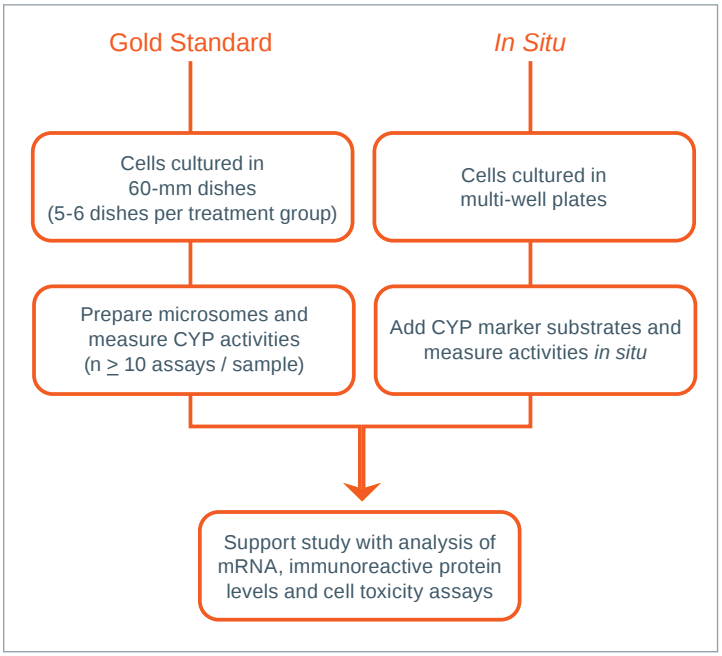
Microsomal Protein Incubations

XenoTech excels in studies designed using the FDA gold standard approach of preparing microsomes from hepatocyte cultures. This study design allows the test article to be washed from the sample before analyzing, comparisons of results between *in vitro* and *ex vivo* test systems, storage of study microsomes for future reference or expanded analysis, and confirmation of results by immunoblotting.

Cell-Based Incubations (in situ)

An alternative approach is using cell based incubations with appropriate marker substrates on treated hepatocyte cultures. XenoTech has the ability to detect the activity of multiple CYP enzymes utilizing *in situ* incubations with the reporter substrate, allowing activity data to be delivered on a per culture basis. XenoTech recommends supplementing activity endpoints with mRNA expression to aid the interpretation of results.

In addition to microsomal analysis and *in situ* incubations, XenoTech can measure mRNA expression levels for CYP450, UGT, transporter protein and other metabolically involved genes through bDNA and RT-PCR technologies. These data can provide valuable insight to the transcriptional effects of an NCE on hepatocyte cultures and can be used to support data derived from enzyme activity analysis.



Enzyme Induction Screen with PCR Arrays

For a comprehensive analysis of the inductive potential of drug candidates, we designed a custom PCR array of 27 human genes regulated by nuclear receptor pathways. The selected genes, which include major inducible CYPs, conjugating enzymes and drug transporters, serve as indicators of the activation of the following nuclear receptor pathways: AhR, CAR, FXR, GR, HNF4a, LXR, Nrf2, PPARa, PXR, and VDR. Every array plate contains two housekeeping genes, reverse transcription, and genomic DNA controls. All genes are analyzed in triplicate. The in-house data demonstrate that the arrays are sensitive and specific. The mRNA analyzed with the microarray is isolated from cultures of primary human hepatocytes, typically treated with three concentrations of the test article for two to three days. In addition to the analysis of the inductive potential of drug candidates with the custom PCR array, we offer analysis of gene expression with standard SABiosciences PCR arrays assembled to answer questions related to drug-induced liver toxicity, signal transduction and mechanisms of drug action.

Ex Vivo Enzyme Induction

Ex vivo induction studies in rat, mouse, dog, monkey or other species can provide valuable information to the investigation of a drug candidate. Not only are these types of induction studies cost-effective, they can provide the following information to your investigation:

- Which prototypical CYP inducers the drug candidate resembles
- Epigenetic tumor formation
- Which species most closely resembles human

β-naphthoflavone, phenobarbital, isoniazid, rifampin, dexamethasone and clofibric acid represent six different CYP enzyme inducers that are useful in studies of *ex vivo* enzyme induction in laboratory animals. Much of our understanding of CYP induction stems from studies conducted in rodents. The design of these studies is generally applicable to other species, although some significant species differences exist, such as:

- Drug-drug interactions
- Pharmacokinetic tolerance
- Increased elimination (auto-induction)

To achieve relevant comparisons, XenoTech maintains a selection of suitable positive controls from rat, mouse, dog and monkey for both CYPs and UGTs to assay with each drug candidate. These positive controls show how your compound relates to the prototypical inducers in your animal model. Additionally, we consult our historical control values acquired over several years to provide the highest level of confidence in our assessment of your compound.

The design of your study will be tailored to your specific needs. We are able to assess CYP and UGT enzymes as well as levels of immunoreactive protein by Western immunoblotting and RT-PCR analysis.

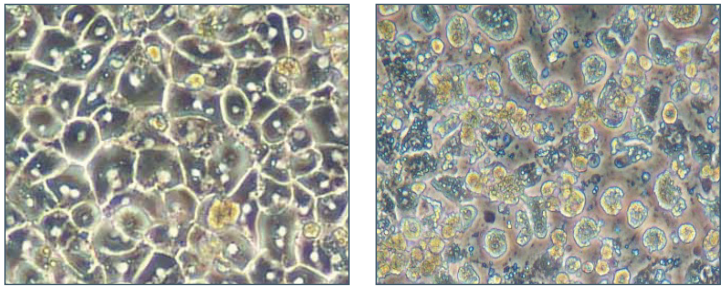
Hepatotoxicity

Hepatotoxicity studies assess the risk that a test article may cause toxicity in humans. These experiments can be performed as a part of your enzyme induction study in human hepatocytes, non-human hepatocytes or immortalized human hepatocytes.

Critical factors that we consider when performing such studies include:

- Strict selection criteria for cultures consisting of morphologically normal cells that have established a confluent monolayer and cell-to-cell contact
- Examining potential toxicity by parent compound and its metabolites
- Analyzing hepatocyte preparations from multiple humans to account for inter-individual variability

It is important to measure endpoints that are well-characterized and understood. We currently use LDH (lactate dehydrogenase)-release assay, Alamar Blue assay and RT-PCR analysis of mRNA. This information is used to generate concentration-response profiles with pharmacologically-relevant concentrations of test articles. In addition, any morphological changes of the cultures are documented with photomicrographs.



Morphological evidence of toxicity observed in human culture H789 treated with 30μM of test article for 72h (right panel) or test article's vehicle (0.1% DMSO, left panel)

Contact us to learn more at **www.xenotech.com** or call **913.438.7450**.

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