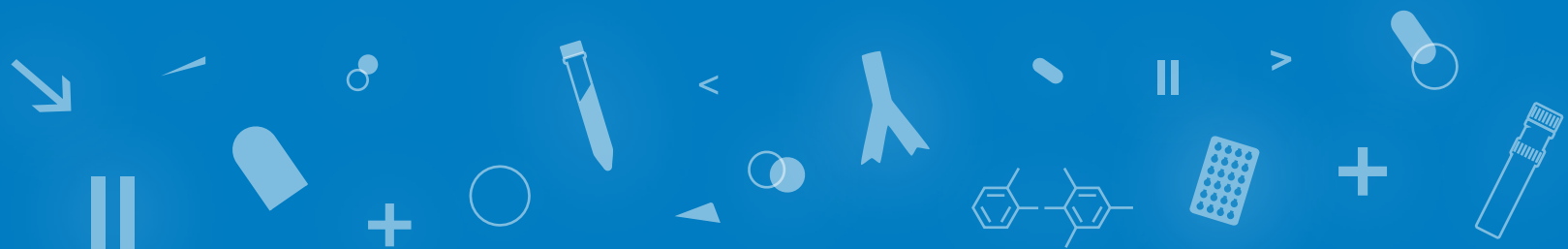
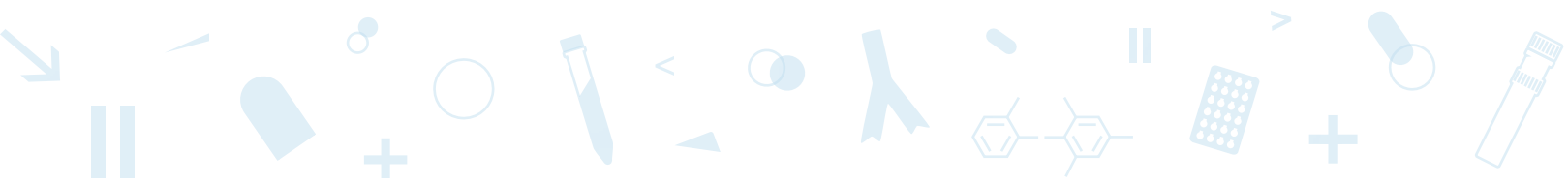




Helping all people  
live healthy lives

# BD Gentest<sup>SM</sup> Contract Research Services





# Partners in the search for new drugs

## Introduction

### BD Gentest<sup>SM</sup> Contract Research Services

BD Gentest Contract Research Services has over 15 years experience developing *in vitro* services to support pharmaceutical drug discovery and development programs in the early ADME/Tox phase. Our Study Directors are highly skilled scientists with in-depth knowledge of absorption and transport, metabolism, and toxicity. This expertise gives BD Biosciences Study Directors the ability to partner with you to develop and deliver a broad range of *in vitro* ADME/Tox studies to meet your discovery and development project needs. We ensure the highest level of quality standards and adhere to current regulatory requirements and applicable FDA-sponsored guidance documents.

Utilizing state-of-the art techniques and equipment, BD Biosciences is able to assist our clients in screening for viable drug candidates during drug discovery or to prepare regulatory agency submission-quality reports for your drug development compounds. Let our team of experts take you to the next level with studies designed to predict drug-drug interactions and human pharmacokinetics using BD Gentest's innovative *in vitro* products, cell models, and methodologies.

#### Acronyms

7-BQ: 7-Benzylxyquinoline	BzRes: 7-Benzylxyresorufin	OCT: Organic cation transporter
7-MFC: 7-Methoxy-4-trifluoro-methyl-Coumarin	CEC: 3-Cyano-7-ethoxycoumarin	OMF: 3-O-Methylfluorescein
ABC: ABC-binding cassette	EFC: 7-Ethoxy-4-trifluoro-methyl-Coumarin	PB: Phenobarbital
ACE: Angiotensin converting enzyme	GLP: Good Laboratory Practice	P-gp: P-glycoprotein
AMMC: 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methyl-Coumarin	HLM: Human liver microsome	PEPT: Proton oligopeptide co-transporter
AZA: Azamulin	KTZ: Ketoconazole	RIT: Ritonavir
AZT: Azidothymidine	MAMC: 7-Methoxy-4-amino methyl-Coumarin	RT-PCR: Real-time reverse-transcription polymerase chain reaction
BCRP: Breast cancer resistance protein	MRP: Multidrug resistance-associated protein	SLC: Solute-linked carrier
BCS: Biopharmaceutics Classification System	NCE: new chemical entity	TEA: Tetraethylammonium
BFC: 7-Benzylxy-4-trifluoro-methyl-Coumarin	NTCP: Sodium taurocholate co-transport protein	TDI: Time-dependent inhibition
BSEP: Bile salt export pump	OAT: Organic anion transporter	UGT: UDP-glucuronosyl transferases
	OATP: Organic anion transporting polypeptide	

## Ordering Information

### United States

#### BD Gentest<sup>SM</sup> Contract Research Services

To discuss and order BD Gentest Contract Research Services, contact BD Biosciences at:

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# Enzyme Inhibition Studies

## Introduction

Inhibition of drug metabolizing enzymes is a major mechanism of drug-drug interactions. For example, one drug may inhibit the metabolism of a co-administered drug, potentially leading to adverse clinical events. Most drug-drug interactions are metabolism based and tied to the cytochrome P450 family of enzymes although many other enzymes may be involved including UGT and transporters. BD Biosciences has over 15 years experience leading development of novel systems and services for *in vitro* enzyme inhibition studies.

## Inhibition of Cytochrome P450 and UGT

The majority of drug-drug interactions are metabolism-based and, of these, most involve cytochrome P450.<sup>1,2</sup> If an NCE is a potent cytochrome P450 inhibitor, it may inhibit the metabolism of a co-administered medication, potentially leading to adverse clinical events.

*In vitro* techniques allow researchers to screen for drug-drug interactions early in the drug development process. BD Gentest<sup>SM</sup> Contract Research Services offers a complete spectrum of cytochrome P450 inhibition tests that combine our industry proven expertise and innovative products to help you identify better drugs faster. Many of these tests can be performed in accordance with GLP regulations.

## High Throughput Cytochrome P450 Inhibition Screening

To expedite identification of drug candidates with cytochrome P450 inhibitory potential, BD Biosciences offers a high throughput screening service. The inhibition of human CYP1A2, 2C8, 2C9, 2C19, 2D6, 3A4, and other isoforms are assessed using BD Supersomes<sup>SM</sup> Enzymes and fluorescence detection methods.<sup>3,4</sup> A comprehensive listing of the enzyme/substrate pairs is shown in the table below. Data are reported as IC<sub>50</sub> values and percent inhibition. LC/MS applications are also available in high throughput format.

Enzyme	Fluorescent Substrate
CYP1A2	3-Cyano-7-ethoxycoumarin (CEC)
CYP2A6	Coumarin
CYP2B6	7-Ethoxy-4-trifluoro-methyl-Coumarin (EFC)
CYP2C8	Dibenzylfluorescein (DBF)
CYP2C9	7-Methoxy-4-trifluoro-methyl-Coumarin (7-MFC) DBF
CYP2C19	3-Cyano-7-ethoxycoumarin (CEC) DBF 3-O-Methylfluorescein (OMF)
CYP2D6	3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methyl-Coumarin (AMMC) 7-Methoxy-4-amino methyl-Coumarin (MAMC)
CYP2E1	7-MFC
CYP3A4	7-Benzyloxyquinoline (7-BQ) 7-Benzyloxy-4-trifluoro-methyl-Coumarin (BFC) 7-Benzyloxyresorufin (BzRes) DBF

Other enzymes are also available, including CYP1A1, 1B1, 3A5, 3A7, and 19 (aromatase). Rat and dog enzymes available on a custom basis.

## Enzyme Inhibition Services

### Cytochrome P450

- High throughput applications for drug discovery
  - LC/MS
  - Fluorometric
- Conventional assays for regulatory submissions
  - Direct or TDI
- Endpoints include:
  - IC<sub>50</sub>
  - IC<sub>50</sub> "shift"
  - K<sub>m</sub>
  - K<sub>i</sub> and k<sub>inact</sub>

### UGT

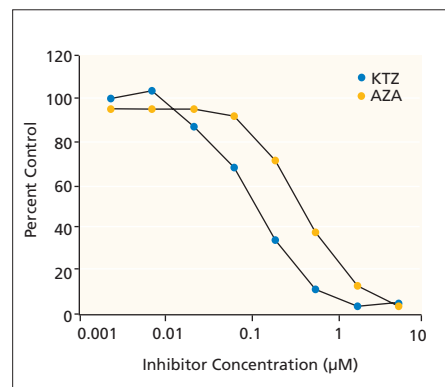
- Endpoints include:
  - IC<sub>50</sub>
  - K<sub>i</sub>

## Custom Designed Studies

## Inhibition of Cytochrome P450 Using Conventional Assays

This test uses human CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 4A11 enzymes, and several model substrates including phenacetin, coumarin, bupropion, paclitaxel, amodiaquine, diclofenac, (S)-mephenytoin, bufuralol, dextromethorphan, *p*-nitrophenol, testosterone, nifedipine, midazolam, and lauric acid. Please inquire as other enzyme substrate pairs may be available. Because inhibition constants are substrate-dependent for CYP3A4, multiple substrates (e.g., testosterone, nifedipine, and midazolam) are available for this enzyme.<sup>5,6</sup> This protocol uses a single substrate concentration near the apparent  $K_m$  and multiple test article concentrations. An  $IC_{50}$  value is defined as the point where 50% inhibition of enzyme catalytic activity occurs and follow up  $K_i$  testing to determine a potent or moderate inhibitor is often conducted. In most assays, we employ the use of BD Gentest™ Stable-labeled Isotope Metabolite Standards to control for ion suppression and potential fluctuation in LC/MS signal response. All tests are developed using state-of-the-art methods and adhere to applicable guidance documents from the FDA.<sup>7-9</sup>

Cytochrome P450 Enzyme	Enzyme Assay	Positive Control Inhibitor
CYP1A2	Phenacetin <i>O</i> -deethylase	7,8-Benzoflavone
CYP2A6	Coumarin 7-hydroxylase	Tranylcypromine
CYP2B6	Bupropion hydroxylase	KTZ
CYP2C8	Amodiaquine N-deethylase Paclitaxel 6 $\alpha$ -hydroxylase	Montelukast
CYP2C9	Diclofenac 4'-hydroxylase	Sulfaphenazole
CYP2C19	(S)-Mephenytoin 4'-hydroxylase	(+)- <i>N</i> -3-Benzylirvanol
CYP2D6	Dextromethorphan N-demethylase	Quinidine
CYP2E1	<i>p</i> -Nitrophenol hydroxylase	4-Methylpyrazole
CYP3A4	Testosterone 6 $\beta$ -hydroxylase Midazolam 1'-hydroxylase Nifedipine oxidase	KTZ
CYP4A11	Lauric acid 12-hydroxylase	17-Octadecanoic acid

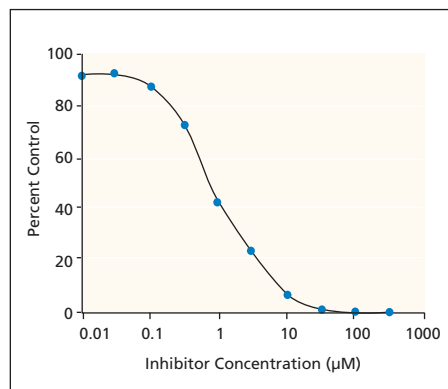


Inhibition of human liver micromes (HLM)-catalyzed midazolam 1'-hydroxylase by KTZ or AZA.

## Time-Dependent Inhibitor (TDI) Testing

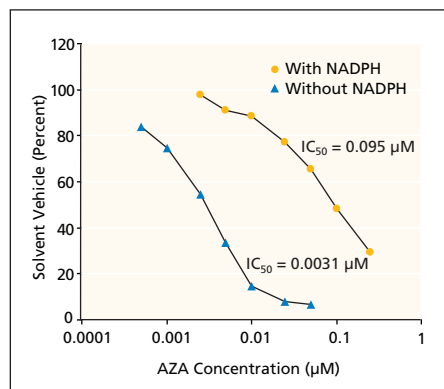
Compounds that fail in the development stage because of toxicity or inappropriate pharmacokinetics are often found to be mechanism-based inhibitors (sometimes referred to as time- and NADPH-dependent inhibitors).<sup>10</sup> Anticipate these problems long before valuable resources are devoted to compounds that eventually may fail. This test determines the time- and NADPH-dependent loss of catalytic activity in liver microsomes. Initial investigations may include  $IC_{50}$  shift assays with follow-up  $K_i$  and  $k_{inact}$  testing. Appropriate positive controls for TDI, such as AZA, tienilic acid, paroxetine, and furafylline are included.

### $IC_{50}$ Determination



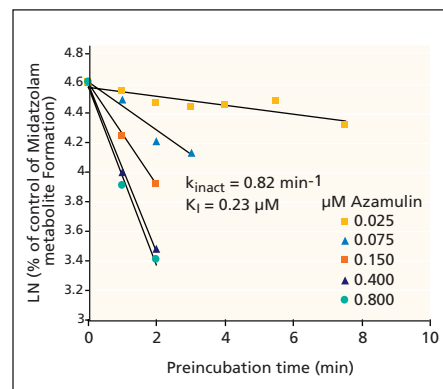
Effect of Drug X on UGT1A1-catalyzed bilirubin glucuronidation.

### Shift in $IC_{50}$ Value After Preincubation with NADPH



Effect of preincubation with and without NADPH on the inhibition of HLM-catalyzed midazolam 1'-hydroxylase assay.

### AZA Inhibition of CYP3A4



Determination of the inactivation rate constant of AZA in the HLM-catalyzed midazolam 1'-hydroxylase assay.

## Inhibition of UGT: $IC_{50}$ Determination

Testing for the inhibition of several UGT isoforms can include UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B15, and 2B17 enzymes using the model substrates 7-hydroxy-trifluoro-methyl-coumarin, 17 $\beta$ -estradiol, trifluoperazine, bilirubin, or eugenol.<sup>11</sup> Please inquire for additional UGT isoforms. This protocol uses a single substrate concentration near the apparent  $K_m$  and multiple test article concentrations (number and spacing are flexible). An  $IC_{50}$  value is determined as the point where 50% inhibition of enzyme catalytic activity occurs. There are several advantages to using cDNA-expressed enzymes for inhibition studies. For example,  $IC_{50}$  values obtained can be compared with clinically significant inhibitors of the same enzyme without the complication of competing pathways of metabolism.

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