

BD GentestSM Contract Research Services

Identify the enzymes responsible for the metabolism of your drug candidate.

Cytochrome P450 Reaction Phenotyping

INTRODUCTION

The number of cytochrome P450 enzymes responsible for the metabolism of a drug affects population variability in metabolism. Drugs cleared metabolically by few enzymes may exhibit susceptibility to co-medication drug interactions or display excessive population variability in metabolism. Reaction phenotyping experiments can identify the number and type of enzymes responsible for drug clearance.

The use of liver microsomes coupled with enzyme selective chemical or antibody inhibitors as well as a panel of cDNA-expressed enzymes provides a robust approach to determine the number and identity of enzymes involved in the metabolism of your test article. The amount of each cDNA-expressed enzyme is chosen to be proportional to the activity of the same enzyme in pooled human liver microsomes (HLM). Protein concentration is standardized by the addition of control microsomes that lack catalytically active enzyme.

Metabolism can be measured by loss of parent compound and/or formation of metabolites. Multiple protocols are available to meet your needs in both discovery and development. HPLC analysis with absorbance, fluorescence, radiometric, or mass spectrometric detection is available. Alternatively, the incubations can be returned to the sponsor for analysis. Our protocols are consistent with the experimental approaches that have been recently reviewed.¹⁻³

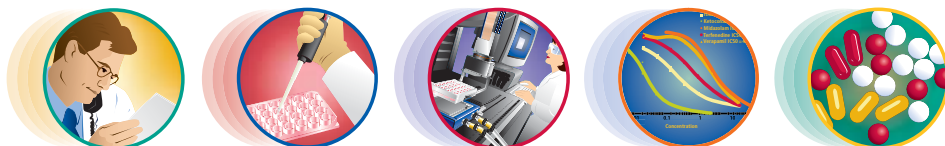
PROTOCOLS AVAILABLE

- **Cytochrome P450 reaction phenotyping – Development**
Test a comprehensive panel of P450 isoforms and FMO3. Quantifying the formation of metabolite rather than loss of parent is preferred to provide the most robust analysis.
- **Cytochrome P450 reaction phenotyping – Discovery**
Test the five major P450 isoforms, CYP1A2, 2C9, 2C19, 2D6, and 3A4. Quantifying loss of parent is preferred to provide a rapid turnaround of results.
- **Uridine diphosphoglucuronosyl transferase reaction phenotyping**
At least 12 UGT enzymes can be examined. Selective chemical inhibition and correlation analysis is available for a subset of these enzymes.
- **Relative role of flavin-containing monooxygenase (FMO) and cytochrome P450 in metabolism**
Heat-inactivation of FMO and non-specific chemical inhibition of P450 to determine the relative contributions of these two oxidative enzyme systems in the metabolism of the test article.
- **Custom designed protocols**
Protocols based upon sponsor requirements.



EXPERIMENTAL OUTLINE

- 1 Determine study design based on client needs.
- 2 Adapt or develop analytical method.
- 3 Time dependence of metabolite formation in HLM.
- 4 HLM protein dependence of metabolite formation.
- 5 Substrate concentration dependence: K_m / V_{max} determination: using HLM as a protein source.
- 6 Test compound incubated with multiple cDNA-expressed enzymes. The concentration of enzyme may be fixed or scaled to mimic the P450 isoform content in pooled liver microsomes.
- 7 Inhibitor analysis: Incubate with and without the chemical/antibody inhibitors.
- 8 K_m / V_{max} determination with cDNA-expressed enzymes involved in the metabolism of the test compound.
- 9 Correlation analysis with a panel of liver microsomes from single donors.



ENZYMES AVAILABLE

CYP1A1	CYP19
CYP1A2	FMO1
CYP1B1	FMO3
CYP2A6	FMO5
CYP2B6	UGT1A1
CYP2C8	UGT1A3
CYP2C9	UGT1A4
CYP2C18	UGT1A6
CYP2C19	UGT1A7
CYP2D6	UGT1A8
CYP2E1	UGT1A9
CYP2J2	UGT1A10
CYP3A4	UGT2B4
CYP3A5	UGT2B7
CYP3A7	UGT2B15
CYP4A11	UGT2B17
CYP4F2	Monoamine oxidase A
CYP4F3a	Monoamine oxidase B
CYP4F3b	N-acetyltransferase 1
CYP4F12	N-acetyltransferase 2

In most cases, only a subset of enzymes need be examined to provide a robust reaction phenotyping analysis. However, some investigations may require screening of the entire list of available enzymes. The list above shows the human BD Gentest™ cDNA-expressed enzymes available for phenotyping analysis as of October 2004.

References

- Williams, J.A., Hurst, S.J., Bauman, J., Jones, B.C., Hyland, R., Gibbs, J.P., Obach, R.S., Ball, S.E. *Reaction phenotyping in drug discovery. Moving forward with confidence?* *Curr. Drug Metab.* **4**:527 (2003).
- Bjornssen, et al., *The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective.* *Drug Metab Dispos.* **31**:815 (2003).
- Lu, A.Y.H., Wang, R.W., and Lin, J.H. *Cytochrome P450 in vitro reaction phenotyping: A re-evaluation of approaches used for P450 isoform identification.* *Drug Metab. Dispos.* **31**:345 (2003).

BD GENTEST SUPERSOMES™ ENZYMES PANEL FOR DRUG X

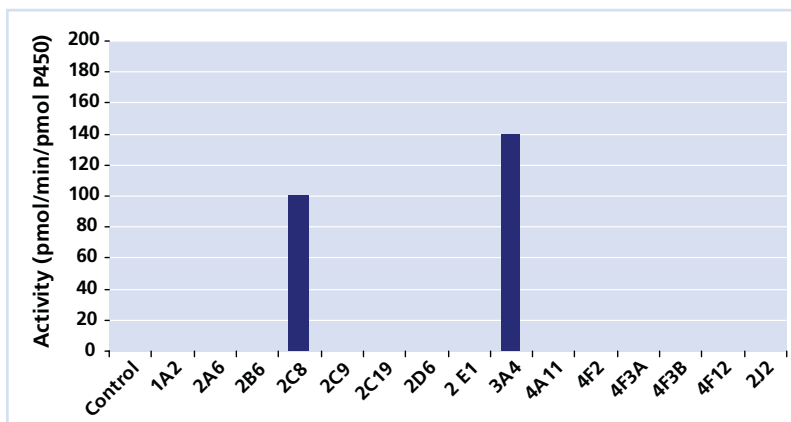


Figure 1: Metabolism of Drug X using BD Supersomes™ Enzymes.

KINETIC ANALYSIS FOR DRUG X (K_M AND V_{MAX})

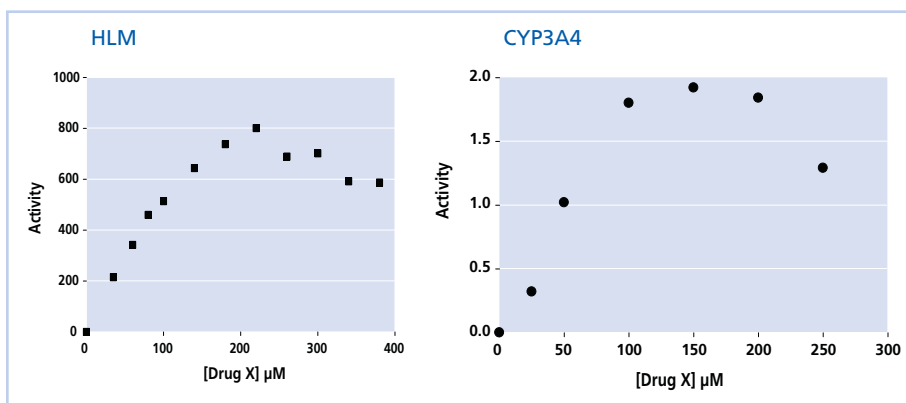


Figure 2: Comparison of enzyme kinetic parameters in HLM and recombinant CYP3A4.

CONTACT INFORMATION

Call the BD Gentest™ Contract Research Services Department at:

888.334.5229 (North America), **781.935.5115** (Worldwide)

E-mail info_gentest@bd.com

Your local BD Gentest™ Product and Services Sales Specialist will promptly provide you with initial study and price information. Your project will be assigned to a Study Director who will coordinate and tailor your Reacton Phenotyping Study to your total satisfaction. A finished detailed report will be provided.

BD Biosciences
Two Oak Park
Bedford, MA 01730 USA
tel: 800.343.2035
fax: 800.743.6200

Nippon BD
Akasaka DS Bldg.
5-26 Akasaka 8-chome
Minato-ku, Tokyo 107 Japan
tel: (81) 24 593 5405
fax: (81) 24 593 5761

BD
2771 Bristol Circle
Oakville, Ontario
Canada L6H 6R5
tel: 905.855.5550
fax: 905.829.5405

BD Biosciences Europe
Erembodegem-Dorp 86
9320 Erembodegem, Belgium
tel: (32) 53 720 211
fax: (32) 53 720 450
e-mail: contact_bdb@europe.bd.com

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale. BD, BD logo, and all other trademarks are the property of Becton, Dickinson and Company. ©2004 BD F04T078



BD

BD Biosciences