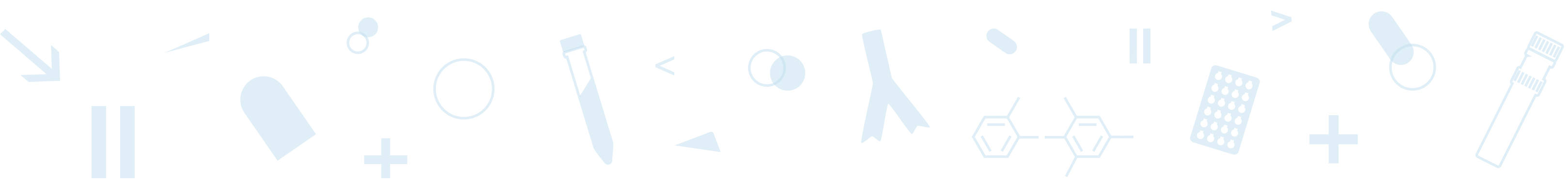
Helping all people
live healthy lives

BD Gentest℠ Contract Research Services



Partners in the search for new drugs

Introduction

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

Ordering Information

United States

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International

Orders for BD Gentest℠ Contract Research Services, should be placed with your regional BD Biosciences office or contact BDBCustomerService@bd.com for further details.

Enzyme Induction Studies

Enzyme Induction Services

- **Human Hepatocyte Models**
  - RT-PCR
  - *In situ* enzyme activity
  - Microsomal enzyme activity
  - Western blotting
- **Preclinical species**
  - *Ex vivo* studies
- **Cytochrome P450 and P-gp**
- **Optional tests include cytotoxicity and solubility in hepatocyte media**

Custom Designed Studies

Introduction

Enzyme induction following drug administration can lead to enhanced clearance of co-medications or itself, causing a drug-drug interaction, therapeutic failure, patient management, and potentially other safety issues.[1-6] *In vitro* models using human hepatocytes can predict the potential of drug candidates to be enzyme inducers. Examination of induction in preclinical species can shed light on the results of animal pharmacokinetic studies and may be predictive of tumorigenicity.

P450 Studies in Human Hepatocytes

BD Biosciences can test several P450 isoforms, including CYP3A4, 2C9, 1A2, 2C19, and 2B6, for induction potential. In this test, your compounds will be exposed to plated fresh or cryopreserved human hepatocytes for three days. Induction can be measured by comparing hepatocyte enzyme activity directly in wells (*in situ*).[7] Alternatively, our scientists can prepare microsomes from hepatocytes and measure the catalytic activity. Western blotting of microsomal or homogenate protein is also available. Finally, for the most rapid turn-around time, total RNA can be extracted from treated hepatocytes and used for the assessment of mRNA expression by quantitative, RT-PCR. Appropriate positive controls for induction are included. All tests are developed using state-of-the-art methods and adhere to applicable guidance documents from the FDA.[8]

RT-PCR Assays for CYP Induction

In this test total RNA extracted from cryopreserved or human fresh hepatocytes treated for two to three days with different inducers is evaluated for CYP mRNA expression by quantitative real-time RT-PCR. RT-PCR and mRNA expression measurements are useful for measuring induction mechanisms and detecting enzyme inducers functioning as enzyme inhibitors by masking the induction response measured by enzyme activity.

This technique is often used in conjunction with microsomal assays as it may not be a measure of enzyme activity. Appropriate positive controls are included.

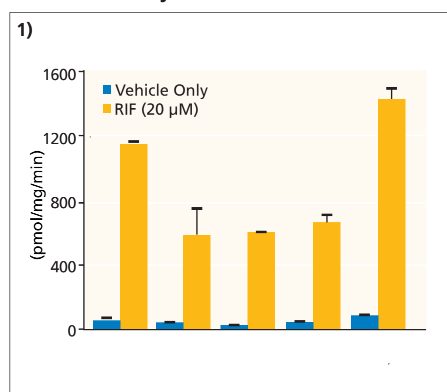
Available Cytochrome P450 enzymes include CYP1A1, 1A2, 2C8, 2C9, 2C19, 3A4, as well as the ABC transporter, P-gp. Appropriate positive controls are used. Custom assays are also available.

In Situ Assays for CYP Induction

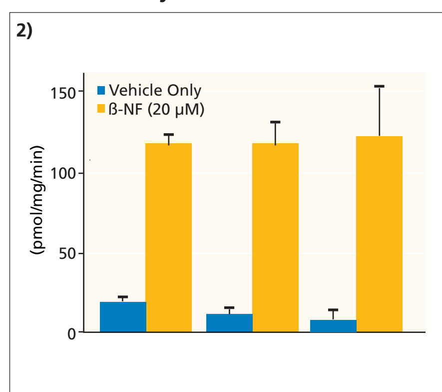
Primary cultured human hepatocytes were treated for three consecutive days with different inducers: 20 μM β-naphthoflavone (β-NF) for CYP1A2, 2 mM phenobarbital (PB) for CYP2B6, and 20 μM rifampicin (RIF) for CYP2C9, 2C19, and 3A4. The expression of CYP enzymes in response to inducers was measured by catalytic activity using probe substrates. The activity of CYP1A2 and 3A4 was determined by measuring *O*-deethylation of phenacetin and 6β-hydroxylation of testosterone, respectively. The catalytic activity of CYP2B6 and 2C19 was determined by analysis of *N*-demethylation and 4-hydroxylation of *S*-mephenytoin, respectively. The measurement of 4-hydroxylation of diclofenac was used to determine the catalytic activity of CYP2C9.

The results (**Figures 1-5**) show the effect of the inducers on CYP induction in hepatocytes isolated from either three (CYP1A2, 2B6, 2C9, and 2C19) or five human donors (3A4) assessed by enzyme activity *in situ*. Treatment with RIF resulted in a 3-fold increase of activity of CYP2C9 and 2C19, and a 17-fold increase of CYP3A4 activity. β-NF and PB increased CYP1A2 and 2B6 activity by approximately 9-fold.

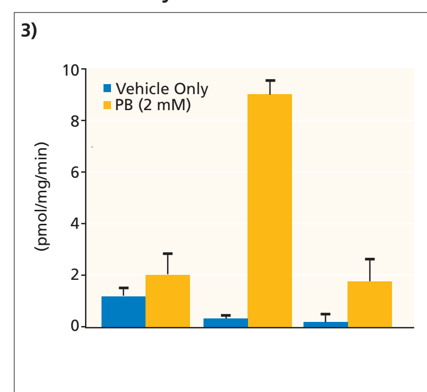
CYP3A4 Activity



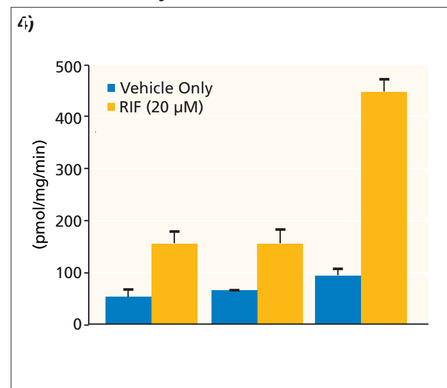
CYP1A2 Activity



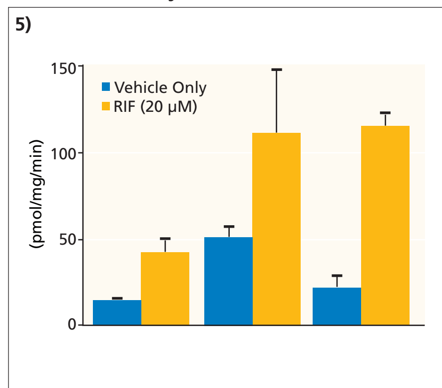
CYP2B6 Activity



CYP2C9 Activity



CYP2C19 Activity



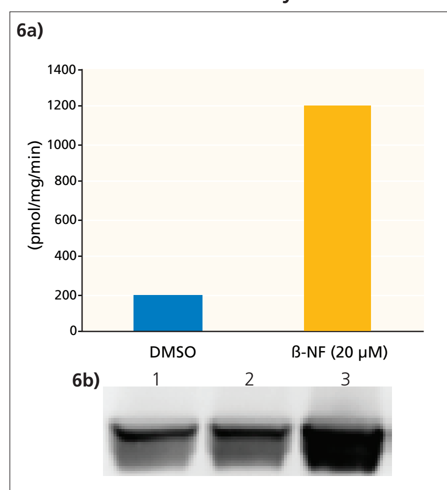
Figures 1-5: *Induction of cytochrome P450 isoforms in primary cultured human hepatocytes isolated from three to five donors. The hepatocytes were incubated with ß-NF, PB, or RIF for 72 hours. After incubation, the catalytic activity of P450 isoforms was determined* in situ. *The bar charts represent three or five donors (CYP3A4 only) and each bar value is the mean of three determinations.*

Microsomal Assays for CYP Induction

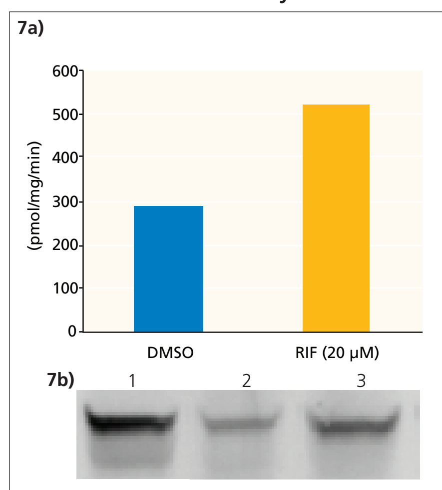
Primary cultured human hepatocytes in 100 mm dishes were treated for three consecutive days with different inducers: 20 µM β-NF for CYP1A2, 20 µM RIF for CYP2C9, and 2 µM RIT for CYP3A4. Microsomes were isolated after treatment and the expression of microsomal CYP enzymes in response to inducers was determined by measuring catalytic activity using probe substrates, as described above, or by Western blotting.

Treatment with β-NF caused a 6-fold induction in microsomal catalytic activity for CYP1A2, and RIF treatment resulted in a 2- and 4-fold induction for CYP2C9 and 3A4, respectively. In contrast, RIT caused a sharp decrease in CYP3A4 activity as expected due to enzyme inactivation. Immunoblotting results showed induction by both β-NF, RIF, and RIT, as expected.

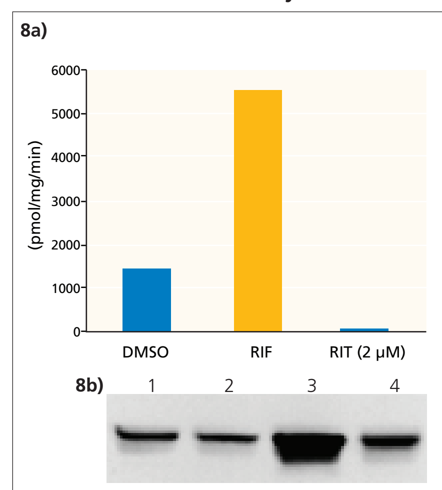
Microsomal CYP1A2 Activity



Microsomal CYP2C9 Activity



Microsomal CYP3A4 Activity



Figures 6-8: *Induction of cytochrome P450 isoforms in microsomes isolated from primary cultured human hepatocytes by β-NF, RIF, or RIT. Hepatocytes were incubated with the inducers for a total of 72 hours. After incubation, the induction of P450 isoforms was determined by measuring microsomal catalytic activity and Western blotting.*

Figures 6-8a: *Catalytic activities measured were phenacetion O-deethylase, diclofenac 4'-hydroxylase, and testosterone 6β-hydroxylase, respectively.*

Figures 6-8b: *Western blot panels–Lane 1: Pooled HLMs (mobility standard); Lane 2: Solvent vehicle control (DMSO); Lane 3: positive control inducer; Lane 4 (CYP3A4 only): RIT.*

Studies in Preclinical Species

Induction of Cytochrome P450 and UGT in Animal Livers (*ex vivo*)

Induction studies in preclinical species may correlate with human induction responses to predict human drug-drug interactions. Cytochrome P450 and UGT induction potential of a drug candidate is evaluated by measuring catalytic activity in livers from preclinical species treated with your compound. The evaluation can include determination of protein yield, total P450 content, cytochrome b_5 content, cytochrome c reductase activity as well as CYP1A1/2, 2B1/2, 2E1, 3A, 4A, and UGT catalytic activities. Induction is measured by catalytic activity within microsomes from treated animals versus vehicle-only treated or untreated animals. Appropriate positive controls for induction are included.

Assays Used for Testing Induction of Enzyme Activity in Preclinical Species

Enzyme	Activity Assay
CYP1A	EROD
CYP2B	Testosterone 16α/β-hydroxylase; BROD
CYP2E1	*p*-Nitrophenol (PNP) hydroxylation, lauric acid 11-hydroxylation
CYP3A	Testosterone 6β-hydroxylation
CYP4A	Lauric acid 12-hydroxylation
UGTs	4-MU, PNP

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