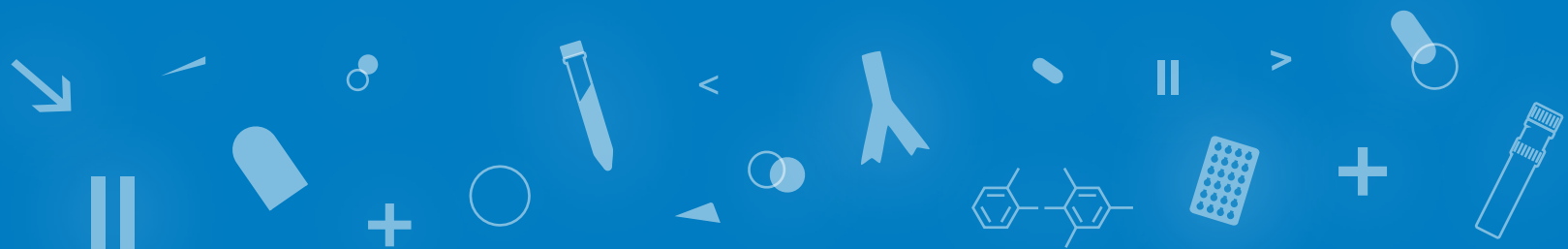
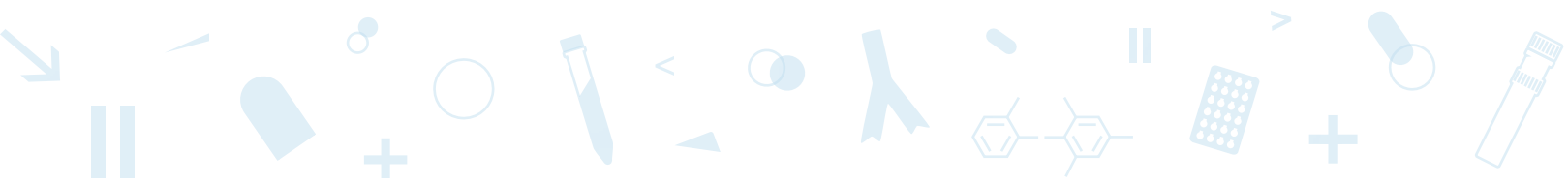




Helping all people
live healthy lives

BD GentestSM Contract Research Services





Partners in the search for new drugs

Introduction

BD GentestSM 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-Benzoyloxyquinoline	BzRes: 7-Benzoyloxyresorufin	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-Benzoyloxy-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 GentestSM Contract Research Services

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

tel: 888.334.5229 x2246 or
781.935.5115 x2246

Technical Support

Contact a BD Biosciences Technical Support Representative at:

tel: 877.232.8995 or 978.901.7389

Monday through Friday

9:00 a.m. – 6:00 p.m. Eastern Time

fax: 978.901.7491

e-mail: admetox@bd.com

International

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

Permeability and Transport Studies

Introduction

Membrane and vesicle transporters play a key role in determining the exposure of liver, kidney, brain, and other tissues to a variety of solutes, including nutrients, cellular by-products, environmental toxins, drugs, and other xenobiotics. Transporters are essential in the delivery and excretory processes of drugs and their metabolites. Drugs that inhibit membrane transporters can alter the absorption, disposition, and elimination of co-administered drugs that are substrates of these transport proteins and can affect bioavailability or cause drug-drug interactions.

BD Biosciences offers a range of contract research services to evaluate drug-transporter interactions using *in vitro* models expressing human and animal transporters from the ABC and SLC superfamilies.

ABC Transporter Services

■ P-gp/MRP/BCRP/BSEP Membrane and Vesicle

- ATPase Assay
- Uptake Assay
- Inhibition Assay

■ MDR1 LLC-PK₁ Cell Model

- Transport Assay
- Inhibition Assay

■ Caco-2 Cell Model

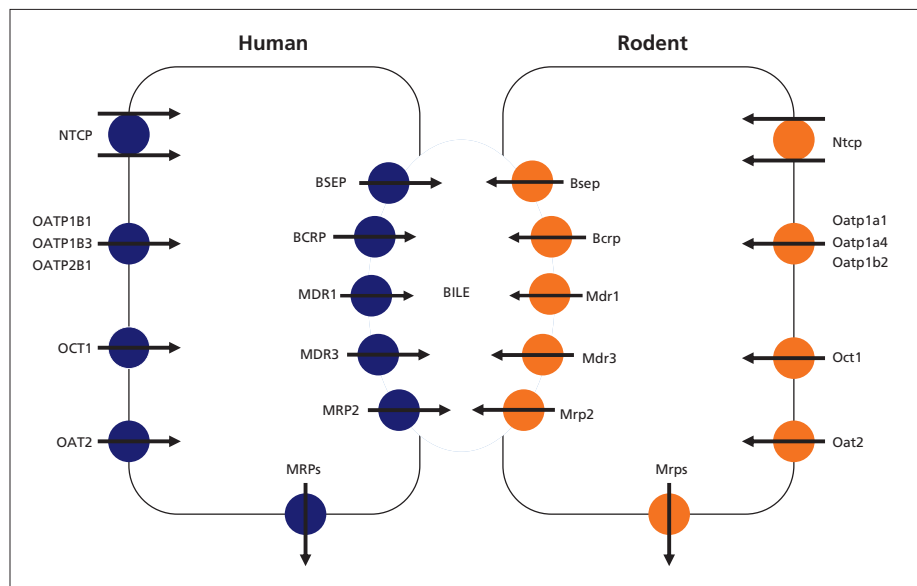
- 21-day and 5-day Monolayers
- Permeability Assay
- Transport Assay
- Inhibition Assay

SLC Transporter Services

■ Transportocytes

- Direct Uptake Assay
- Inhibition Assay

Custom Designed Studies



An "idealized" cell showing the mechanism and direction of transport for many influx and efflux transporters available from BD Biosciences.

ABC Transporter Services

Epithelial cell barrier systems using Caco-2 or LLC-PK₁ cells are widely accepted *in vitro* models used to rank order absorption of drug candidates. In addition to these standard models used for drug absorption, BD Biosciences offers unique capabilities to specifically study human P-gp-mediated drug transport using cDNA transfected LLC-PK₁ porcine cell lines. These human P-gp expressing cell lines offer the advantage of studying this important efflux transporter without interference from other expressed transporters.

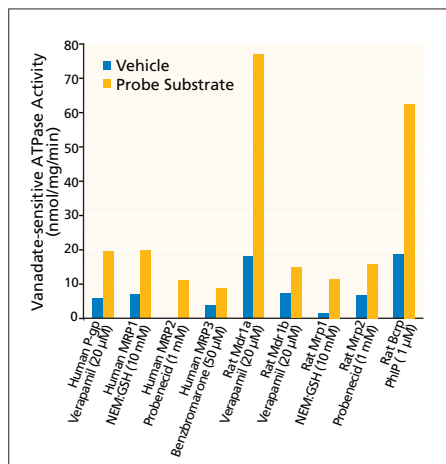
Service	Format	Species	Application
ABC Transporter Screening	Insect cell membranes expressing transporter cDNA (P-gp, MRP, BSEP, BCRP)	Human, Monkey (rhesus, cynomolgus), Dog, Rat (1a and b), Mouse (1a and b)	ATPase assay to identify potential substrates; Discovery
ABC Transporter Interaction	Direct measurement of transport using ABC inside-out transporter vesicles	Human MRP1, MRP2, MRP3, Rat Mrp1, Mrp2, Rat Bsep, Human BCRP, Rat Bcrp, Mouse Bcrp	Substrate and inhibitor identification; Discovery and Development
P-gp (MDR1) Interaction Characterization	Bi-directional transport measurement in polarized cell monolayers (Caco-2, LLC-PK ₁)	Human MDR1, Monkey (cynomolgus) Mdr1	Substrate and inhibitor determination; Discovery P-gp-mediated drug-drug interaction assessment, K_m/V_{max} , IC_{50} , K_i ; Development

ABC Transporter Screening

ATPase Assay in Membranes

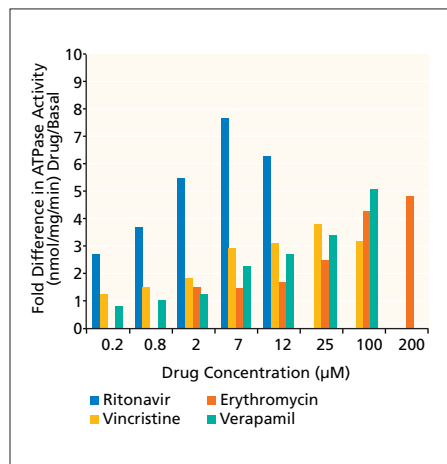
Determine if your compounds interact with ABC transporters. ATP hydrolysis is required for *in vivo* drug efflux by ABC transporters. The membrane ATPase assay measures the phosphate liberated from drug-stimulated ATP hydrolysis in BD Gentest™ human and animal ABC Transporter Membranes.

Probe Substrate Activity in ABC Transporter Membranes



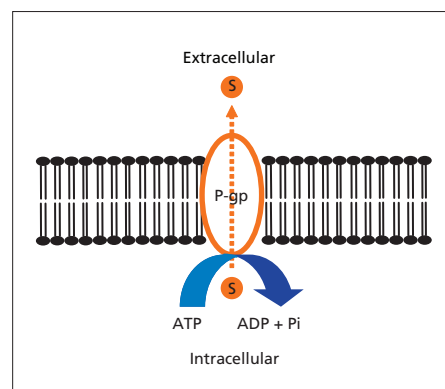
Drug-stimulated, vanadate-sensitive ATPase activity in human and animal ABC transporter membranes.

ATPase Activity of Selected Compounds in Human P-gp Membranes



Concentration dependence of drug-stimulated ATPase activity in human P-gp membranes by MDR1 substrates.

ATPase Model



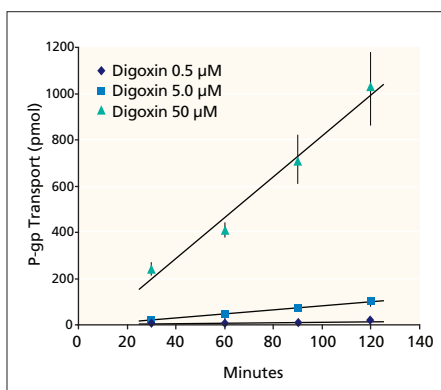
P-gp Interaction Characterization

Drug Transport in Polarized Cell Monolayers

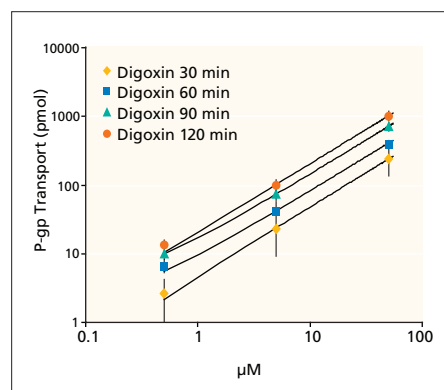
P-gp, encoded by *MDR1*, is a member of the ABC transporter superfamily and is expressed in the human intestine, liver, brain, and other tissues. Localized to the cell membrane, P-gp functions as an ATP-dependent efflux pump, capable of transporting many structurally unrelated xenobiotics out of cells. Intestinal expression of P-gp may affect the oral bioavailability of drug molecules that are substrates for this transporter. Determine if your compounds are P-gp substrates by a direct measure of transport across polarized cell monolayers. Bidirectional transport (apical to basolateral and basolateral to apical) is measured in Caco-2 cells, or in LLC-PK₁ cells expressing P-gp cDNA and corresponding control cells. Radiochemical or LC/MS analysis is available. Alternatively, the incubations can be returned to the Sponsor for analysis. Controls for membrane integrity and comparator compounds are included.

BD Biosciences has developed LLC-PK₁ cell lines expressing human and monkey P-gp. The BD Gentest™ *MDR1*-LLC-PK₁ Cell Model consists of cell monolayers expressing P-gp cDNA, and control (vector bearing) cell monolayers. This model provides P-gp-specific transport and inhibition data. Evaluation of your lead drug candidate as a substrate and inhibitor of P-gp is performed according to FDA guidance.³

P-gp-Facilitated Transport Time Dependence



P-gp-Facilitated Transport Concentration Dependence

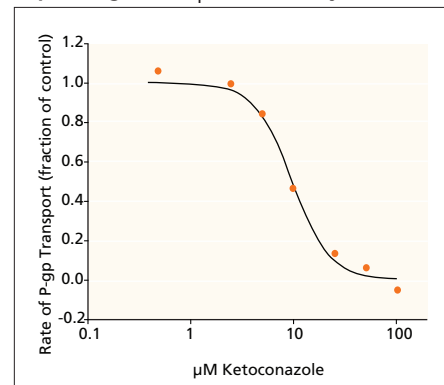


Transport results obtained in the *MDR1*-LLC-PK₁ cell model, demonstrating that the P-gp-facilitated transport of digoxin is time and concentration dependent.

Inhibition of P-gp-Mediated Drug Transport in Polarized Cell Monolayers

Drugs that inhibit P-gp can alter the absorption, disposition, and elimination of co-administered drugs and can enhance bioavailability or cause unwanted drug-drug interactions. Determine if your compounds inhibit P-gp-mediated transport of a model substrate across polarized cell monolayers. Typically, multiple test compound concentrations are examined. Data are reported as IC₅₀ values or percent inhibition. Positive controls for P-gp inhibition and membrane integrity are included.

Inhibition of P-gp-Mediated Drug Transport by Selected Chemicals in Human P-gp Expressing LLC-PK₁ Cell Monolayers



Inhibition results obtained in the *MDR1*-LLC-PK₁ cell model, demonstrating the concentration dependence of digoxin transport inhibition by ketoconazole.

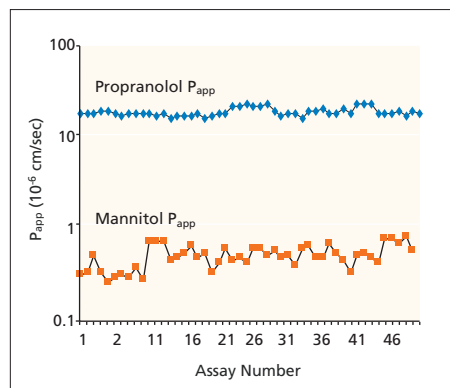
Drug Permeability and Transport Measurement in Caco-2 Cell Monolayers

Drug permeability through cell monolayers correlates well with intestinal permeability and oral bioavailability. Several mammalian cell lines are appropriate for this measurement. Apical to basolateral diffusion is measured using a standard set of time points and drug concentrations or adapted to a high throughput mode. Analysis is by LC/MS detection. Alternatively, the incubations can be returned to the Sponsor for analysis. FDA-recommended permeability comparators and controls for membrane integrity are included. Data are reported as apparent permeability (P_{app}) or percent flux values under fixed conditions.

Compound	Apical to Basolateral P_{app} Value ($\times 10^{-6}$ cm/sec)	% Human Absorption*
Erythromycin	0.53	–
Vinblastine	0.62	poor
Mannitol	0.63	15
Atenolol	0.33	44
Cimetidine	0.75	62
Cyclosporin A	1.4	40
Digoxin	1.3	66-90
Indinavir	3.6	60-70
Paclitaxel	3.4	–
Ritonavir	7.4	60-70
Verapamil	21	90
Propranolol	24	90
Azidothymidine (AZT)	28	100
Testosterone	36	100
Dexamethasone	40	100

*Values from multiple literature sources.

Summary of P_{app} Values for Propranolol and Mannitol over a Series of Assays



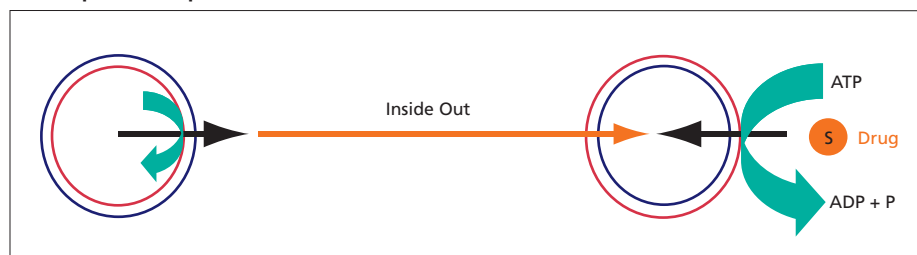
Interassay reproducibility of P_{app} values for FDA Biopharmaceutics Classification System (BCS) permeability comparators.

ABC Transporter Interaction

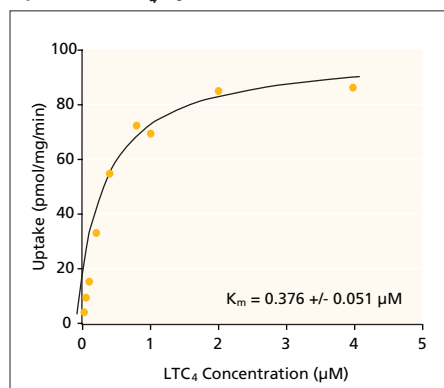
Functional Assays in ABC Transporter Vesicles

ABC inside-out transporter vesicles are used in *in vitro* direct measurement assays to evaluate whether a drug candidate is a substrate or inhibitor of MRP, BSEP, or BCRP transporters. Assays using ABC transporter vesicles directly measure the ATP-dependent transporter-mediated uptake of drug compounds. This information can help predict the interactions between drug compounds and efflux transporters. ABC transporter vesicles are an ideal transport model over cell-based assays for compounds unable to penetrate the plasma membrane by simple diffusion.

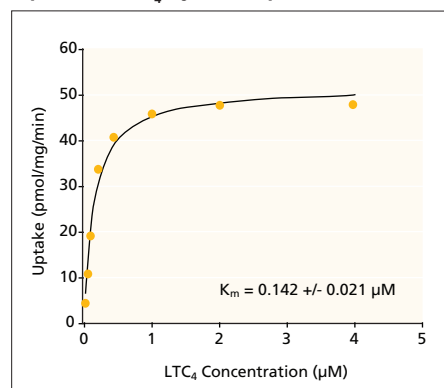
ATP-dependent Uptake Model



Uptake of LTC₄ by Human MRP1



Uptake of LTC₄ by Rat Mrp1



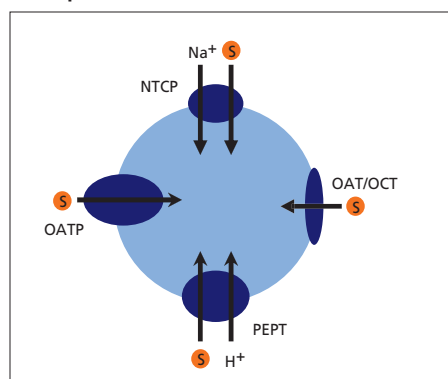
Uptake results obtained in transporter vesicles demonstrating concentration dependence of LTC₄ uptake by human MRP1 and rat Mrp1.

SLC Transporter Services

SLC Transporters Expressed in *Xenopus laevis* Oocytes

SLC membrane transporters play a key role in the uptake of a wide variety of solutes into various tissues. BD Gentest™ Transportocytes are *Xenopus laevis* oocytes pre-injected with OAT, OATP, OCT, NTCP, and PEPT transporter cRNA. *Xenopus* oocytes efficiently express these membrane-bound transporters and, together with control (uninjected or water-injected) oocytes, can be used to predict drug disposition, drug clearance, and drug-drug interactions.

SLC Uptake Model



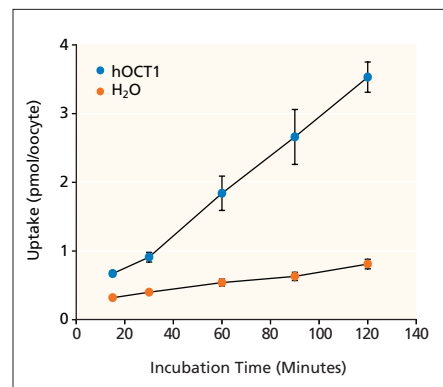
Service	Format	Species	Application
SLC Transporter Interaction Screen	Uptake assays performed in Transportocytes expressing OAT, OATP, OCT, NTCP, PEPT	Human, Rat, Monkey (cynomolgus, rhesus)	Substrate and inhibitor determination; Discovery
SLC Transporter Interaction Characterization			Compound affinity determination (K_m , V_{max} , IC_{50} , K_i), transporter-mediated drug-drug interaction assessment; Development

Transporter-mediated Drug Uptake in *Xenopus laevis* Oocytes

The oocyte expression system is used extensively to study the function of membrane proteins, such as transporters, channels, and pumps because of its low background, high expression level, and proper post-translational modifications. Our data indicates transporters are highly functional when expressed in oocytes. The measured kinetic parameters are repeatable and comparable to published results. Oocytes with high expression of human transporters are used by pharmaceutical companies in lead optimization and drug development to predict drug disposition, drug clearance, and drug-drug interactions.

Transporter	Gene	Main Tissue Distribution	Main Substrates
Human NTCP	<i>SLC10A1</i>	Liver, Pancreas	Cholate, taurocholate, glycocholate, and other bile salts
Human PEPT1 Human PEPT2	<i>SLC15A1</i> <i>SLC15A2</i>	Intestine, Kidney, Lung, Brain	Di- or Tripeptides. Peptide mimetic drugs, such as ACE inhibitors, β -lactam antibiotics, anticancer drugs, and antiviral drugs
Human OATP1A2 (hOATP1) Human OATP1B1 (hOATP2) Human OATP1B3 (hOATP8) Rat Oatp1a1 (rOatp1) Rat Oatp1a4 (rOatp2) Rat Oatp1b2 (rOatp4) Cyno monkey Oatp1b3 Rhesus monkey Oatp1b3	<i>SLC01A2 (SLC21A3)</i> <i>SLC01B1 (SLC21A6)</i> <i>SLC01B3 (SLC21A8)</i> <i>Slco1a1 (Slc21a1)</i> <i>Slco1a4 (Slc21a5)</i> <i>Slco1b2 (Slc21a10)</i> <i>Slco1b3</i> <i>Slco1b3</i>	Brain Liver Liver Liver, Kidney, Brain Liver, Kidney, Brain Liver Liver Liver	Conjugated and unconjugated bile salts, steroids and steroid conjugates, organic compounds, and thyroid hormones
Human OCT1	<i>SLC22A1</i>	Liver, Kidney, Brain, Intestine	TEA, NMN, MPP, and many other organic cations
Human OAT1 Human OAT3 Rat Oat1 Rat Oat2 Rat Oat3	<i>SLC22A6</i> <i>SLC22A8</i> <i>Slc22a6</i> <i>Slc22a7</i> <i>Slc22a8</i>	Kidney, Brain Kidney, Liver, Brain Kidney, Brain Liver, Kidney Kidney, Liver, Brain	Antibiotics, antiviral drugs, non-steroidal anti-inflammatory drugs, and other organic anions

Uptake is Time Dependent

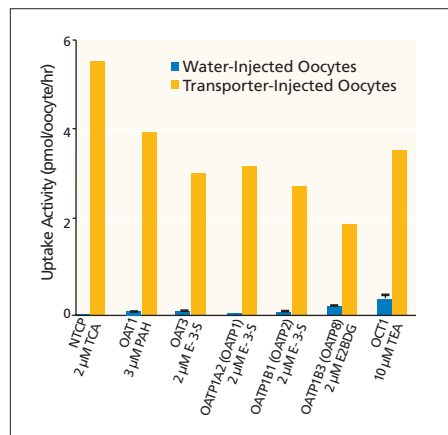


hOCT1: Uptake of 10 μ M TCA

Inhibition of Transporter-mediated Drug Uptake in *Xenopus laevis* Oocytes

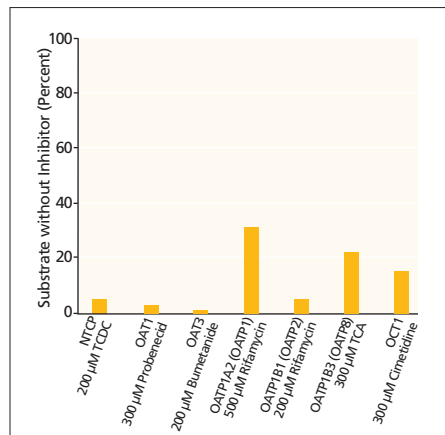
Inhibition of transporter-mediated drug uptake can be measured with indirect assays. In this test, we determine if your compounds can inhibit the transporter-mediated uptake of a model substrate. Typically, multiple test substance concentrations are examined. Data are reported as IC₅₀ values, or percent inhibition when using only one or two concentrations of test compound.

Uptake Activity in Transportocytes



Uptake activity of model probe substrates in control (water-injected) and transporter-injected oocytes.

Inhibition Activity in Transportocytes



Inhibition of transporter-specific uptake of model probe substrates by known inhibitors in transportocytes.

Publications

1. U.S. FDA/CDER, Biopharmaceutics Classification System (BCS), August 2000.
2. Polli, J.W., Wring, S.A., Humphreys, J.E., Huang, L., Morgan, J.B., Webster, L.O., and Serabjit-Singh, C.S. Rational Use of *in vitro* P-glycoprotein Assays in Drug Discovery. *J. Pharmacol. Exp. Ther.* **299**:620 (2001).
3. U.S. FDA/CDER, Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling, DRAFT GUIDANCE, September 2006.
4. Fox, L., Steimel, D., and Crespi, C.L. Heterologous Expression of and the Development of Assays for Interaction of Drugs with Human P-Glycoprotein (P-gp). *Drug Metab. Rev.* **32**(suppl2):197 (2000).
5. Sandage, B., Ph.D., Sabounjian, L., R.N., Shipley, J., M.D., Profy, A., Ph.D., Lasseter, K., M.D., Fox, L., and Harnett, M., M.S. Predictive Power of an *in vitro* System to Address Drug Interactions of an Antimuscarinic Medication: A Comparison of *in vitro* and *in vivo* Drug-Drug Interaction Studies of Trospium Chloride with Digoxin. *J. Clinical Pharmacol.* **46**:1 (2006).



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