SEKISUI XENOTECH

Sekisui XenoTech offers the widest portfolio of transporter services and assays available.

It has been formally acknowledged by regulatory and scientific communities that transporters can play a major role in drug safety issues and should be included in new drug candidate preclinical assessment. Sekisui XenoTech has a multi-pronged study approach to elucidate the drug candidate's interaction with a variety of transporters. Our laboratories in the US and Japan (Sekisui ADME Tox Research Institute) allow us to offer the widest portfolio of drug transporter studies available, including the transporters recommended by the FDA and EMA in the recommended test systems, numerous atypical transporters in stably transfected or transiently transfected cell lines or oocytes and *in vivo* capabilities. We also have the ability to quickly develop assays for new transporters.

Efflux (ABC) Transporters

P-gp (MDR1/ABCB1), BCRP (ABCG2) and BSEP (sPgp/ABCB11) are members of the ATP-binding cassette superfamily, are expressed in the luminal membrane of enterocytes, endothelial cells in the brain, brush border membrane of renal proximal tubules and canalicular membrane of hepatocytes where they limit intestinal absorption limit, blood-brain barrier penetration and facilitate excretion into the bile and urine. BSEP is mainly expressed in the canalicular membrane of hepatocytes where it facilitates excretion into the bile. To evaluate if a test compound is a substrate of P-gp or BCRP, the bidirectional permeability of the test compound across a monolayer of cells (MDCKII-MDR1 or MDCKII-BCRP) in a transwell plate is measured. Inhibition is evaluated by measuring the ability of the test compound to reduce the bidirectional permeability of a probe substrate across Caco-2 (P-gp) or MDCKII-BCRP cells. Accumulation of a test compound in BSEP expressing vesicles is measured to evaluate if the compound is a substrate of BSEP and inhibition is evaluated by measuring the effect of the test compound on the accumulation of a probe substrate.

Hepatic & Renal SLC Transporters

OATP1B1 (OATP-C/OATP2/SLCO1B1), OATP1B3 (OATP-8/SLCO1B3) and OCT1 (SLC22A8) are expressed on the sinusoidal membrane of hepatocytes and facilitate the uptake of endogenous and xenobiotic compounds into hepatocytes followed by metabolism or excretion into the bile. OAT1 (SLC22A6), OAT3 (SLC22A8) and OCT2 (SLC22A2) are expressed on the basolateral membrane of renal proximal tubules and facilitate the uptake of compounds into the proximal tubule which are then excreted in the urine. MATE1 (SLC47A1) and MATE2-K (SLC47A2) are expressed on the brush-border membrane of proximal epithelial cells in the kidney and canalicular membrane of hepatocytes where they efflux organic compounds for further excretion into the urine or bile. Accumulation of a test compound in transporter expressing and control cells (HEK293) is measured to evaluate if the test compound is a substrate of the transporter and inhibition is evaluated by measuring the effect of the test compound on the accumulation of a probe substrate in the transporter expressing and control cells

Guidance Recommendations

	FDA Guidance 2012 ITC Paper 2010[1]	EMA Guidance 2013[2]	PMDA Guidance 2014[3]
P-gp / MDR1	✓	✓	✓
BCRP	✓	✓	✓
BSEP	✓*	✓*	
OATP1B1	✓	✓	✓
OATP1B3	✓	✓	✓
OAT1	✓	✓	✓
OAT3	✓	✓	✓
OCT1		✓*	
OCT2	✓	✓	✓
MATE1	✓*	✓*	✓
MATE2-K	✓*	✓*	✓

* Should be considered

[1] [FDA] Food and Drug Administration: Draft Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing and Labeling Recommendations. Rockville, MD. 2012
International Transporter Consortium, Giacomini et al., Membrane transporters in drug development. Nat Rev Drug Discov. 2010 Mar; 9(3):215-36.

[2] [EMA] European Medicines Agency: CPMP/EWP/560/95/Rev. 1 - Corr. Guideline on the Investigation of Drug Interactions. London, England. 2013

[3] [PMDA] Pharmaceuticals and Medical Devices Agency: Drug Interaction Guideline for Drug Development and Labeling Recommendations (final draft). 2014.

Features of Sekisui XenoTech Transporter Studies

- GLP and non-GLP services are available
- Multiple test systems for the major transporters, from membranes to *in vivo* models
- The most extensive selection of transporters and assays available in the world
- Expert guidance for compound development custom-designed for your needs
- Medium-throughput screening services are available
- Communication and review of all study materials performed by a transporter specialist at Sekisui XenoTech
- Sekisui XenoTech's in-house LC-MS/MS (Analytical), quality assurance and knowledge management groups ensure fast, quality data and reporting

Benefits of Having Sekisui XenoTech As a Partner

- Save time and money with guidance from our experts
- Access to a global team of transporter experts
- Customized studies and reports based on the specific needs for content, turnaround and cost
- Take your program from screening to regulatory submissions
- Full suite of DMPK services including induction, inhibition, metabolism and transporter studies

1101 W Cambridge Cir Dr | Kansas City, KS 66103 | *p* (877) 588-7530 / +913.438.7450 | *f* (913) 227-7100 | xenotech.com | info@xenotechllc.com

Portfolio of Available Drug Transporter Assays

	Cell Lines	Vesicle	Double Transfected	Oocyte	Hepatocyte	Animal Model
P-gp (MDR1)	LLC-PK1, MDCKII, Caco-2	✓				
MRP1		✓				
MRP2		✓	OATP1B1			
MRP3		✓				
MRP4		✓				
BCRP	LLC-PK1, MDCKII, Caco-2	✓				
BSEP		✓				
OATP1A2				✓		
OATP1B1	HEK293		MRP2	✓	✓	
OATP1B3	HEK293			✓	✓	
OATP2B1	HEK293			✓		
OAT1	HEK293, S_2			✓		
OAT2	S_2					
OAT3	HEK293, S_2			✓		
OAT4	HEK293, S_2			✓		
OAT7	HEK293					
OCT1	HEK293, S_2			✓		
OCT2	HEK293, S_2			✓		
OCT3	HEK293, S_2					
OCTN1	S_2					
OCTN2	S_2					
OST α/β	HEK293					
PEPT1	HEK293					
PEPT2	HEK293					
URAT1	HEK293					
NTCP1	HEK293			✓	✓	
NTCP2 (ASBT)	HEK293					
MATE1	HEK293					
MATE2-K	HEK293 (TEA & Metformin)					
NPT1	HEK293					
ENT1	MDCKII					
ENT2	MDCKII					
ENT4	MDCKII					
CNT1	MDCKII					
CNT2	MDCKII					
CNT3	MDCKII					
GLUT1				✓		
GLUT2				✓		
Mouse Mdr1a	LLC-PK1					KO animal
Mouse Mdr1b	LLC-PK1					KO animal
Rat Mdr1a	LLC-PK1					KO animal
Rat Mdr1b	LLC-PK1					
Rat Urat1	HEK293			✓		
Rat Oatp4/Oatp1b2				✓		
Dog Mdr1	LLC-PK1					
Cynomolgus Mdr1	LLC-PK1					
Rhesus Mdr1	LLC-PK1					

Kinetic Parameters of Efflux and Uptake Transporter Assays

Description of construct	Parental Cell line	Gene	Substrates	Kinetic parameters	Kinetic parameters for inhibitors
MDR1	LLC-PK1	ABCB1	1 μM Digoxin	Km: >100 μM	IC50 Verapamil :1.6 μM
					IC50 Cyclosporin A: 0.94uM
BCRP	LLC-PK1	ABCG2	10 nM Prazosin	KM: 19.6 μM	IC50 Ko143: 0.0344 μM
	Vesicle		1 μM Estrone sulfate	KM: 6.77 μM (14.2)	IC50 Ko143: 0.0186 μM (0.026)
BSEP	Vesicle	ABCB11	2 μM Taurocholic acid	KM: 19.1 μM	IC50 Cyclosporine A: 6.68 μM
MRP2	Vesicle	ABCC2	0.1 μM Leukotriene C_4	KM: 5.79 μM (0.24-5.9)	IC50 BSP: 10.0 μM
					IC50 Benzbromarone: 11.1 μM (7.3-8.3)
OATP1B1	HEK	SLCO1B1	50 nM Estradiol glucuronide	KM: 3.94 μM (3.7-8.3)	IC50 Rifampicin: 0.243 μM(0.94)
					IC50 Cyclosporin A: 0.215 μM
OATP1B3	HEK	SLCO1B3	50 nM Estradiol glucuronide	KM: 26.5 μM (5-25)	IC50 Rifampicin: 0.388μM (1.5)
					IC50 Cyclosporin A: 0.386 μM
			20 nM CCK-8	KM: 18.6 μM	IC50 Rifampicin: 1.06 μM
					IC50 Cyclosporin A: 0.612 μM
OATP2B1	HEK	SLCO2B1	50 nM Estrone sulfate	KM: 14.5 μM (7-21)	IC50 Rifampicin: 20.6μM (90)
					IC50 Cyclosporin A: 1.05 μM
OAT1	HEK	SLC22A6	1000 nM p-Aminohippurate	KM: 11.9 μM (4-22)	IC50 Probenecid: 4.70 μM (6.3-12.5)
					IC50 Indomethacin: 1.95 μM
	S_2		1000 nM p-Aminohippurate	KM: 24.6 μM (4-22)	IC50 Probenecid: 11.7 μM (6.3-12.5)
OAT2	S_2	SLC22A7	50 nM PGF2α	KM: 0.137 μM (0.4-0.8)	IC50 Mefenamic acid: 11.7 μM (21.7)
OAT3	HEK	SLC22A8	50 nM Estrone sulfate	KM: 22.8 μM (2-7)	IC50 Probenecid: 2.70 μM (4-30)
					IC50 Cimetidine: 264 μM
	S_2		50 nM Estrone sulfate	KM: 1.59 μM (2-7)	IC50 Probenecid: 5.33 μM (4-30)
OAT4	HEK	SLC22A11	50 nM Estrone sulfate	KM: 15.8 μM (1-10)	IC50 BSP: 0.377 μM (<5)
	S_2		50 nM Estrone sulfate	KM: 7.42 μM (1-10)	IC50 Probenecid: 81.8 μM (25.4)
OAT7	HEK	SLC22A9	50 nM Estrone sulfate	KM: 12.9 μM (8.7-40)	IC50 BSP: 0.121 μM
OCT1	HEK	SLC22A1	5 μM Tetraethylammonium bromide	KM: 576 μM (160-1600)	IC50 Quinidine: 4.57 μM (5.4-23.4)
					IC50 Verapamil: 0.272 μM
			10 μM Metformin	KM: 899 μM	IC50 Quinidine: 13.8 μM
	S_2		5 μM Tetraethylammonium bromide	KM: 453 μM (160-1600)	IC50 Quinidine: 17.9 μM (5.4-23.4)
OCT2	HEK	SLC22A2	10 μM Metformin	KM: 3600 μM (990)	IC50 Quinidine: 88.6 μM (1 mM 5-20%)
			5 μM Tetraethylammonium bromide	KM: 723 μM	IC50 Quinidine: 93.7 μM
					IC50 Verapamil : 192 μM
	S_2		10 μM Metformin	KM: 12500 μM (990)	IC50 Quinidine: 104 μM
			5 μM Tetraethylammonium bromide	KM: 35.1 μM	IC50 Verapamil : 298 μM
OCT3	HEK	SLC22A3	5 μM Tetraethylammonium bromide	KM: 536 μM (+)	IC50 Verapamil: 6.21 μM (24)
					IC50 Quinidine : 12.7 μM
	S_2		5 μM Histamine	KM: 261 μM(180-420)	IC50 Verapamil: 11.6 μM (24)
OCTN1	S_2	SLC22A4	5 μM Tetraethylammonium bromide	KM: 315 μM (199-1280)	IC50 Verapamil: 12.3 μM (8.4)
OCTN2	S_2	SLC22A5	30 nM Carnitine	KM: 4.73 μM (2-66)	IC50 Verapamil: 5.19 μM (500 μM 2.4%)
NTCP1	HEK	SLC10A1	50 nM Taurocholate	KM: 10.2 μM (6.2-10)	IC50 Cyclosporin A: 1.20 μM (1)
NTCP2 (ASBT)	HEK	SLC10A2	200 nM Taurocholate	KM: 15.8 μM (9.4-20)	IC50 Chenodeoxycholic acid: 3.48 μM (Ki 3.3)
NPT1	HEK	SLC17A1	50 nM Estradiol glucuronide	KM: 6.68 μM	IC50 Indomethacin: 2.17 μM
					IC50 Probenecid : 69.1 μM
PEPT1	HEK	SLC15A1	100 nM Glycylsarcosine	KM: 556 μM (290-3130)	IC50 Cephalexin: 1460 μM (5200-13700)
PEPT2	HEK	SLC15A2	100 nM Glycylsarcosine	KM: 59.4 μM (74)	IC50 Cephalexin: 124 μM (10mM 0%)
URAT1	HEK	SLC22A12	50 μM Uric acid	KM: 1050 μM (371)	IC50 Benzbromarone: 0.0334 μM (0.3)
					IC50 Probenecid : 80.0 μM
MATE1	HEK	SLC47A1	10 μM Metformin	KM: 73.4 μM (220)	IC50 Cimetidine: 1.32 μM (<20)
			5 μM Tetraethylammonium bromide	KM: 29.9 μM	IC50 Cimetidine : 1.10 μM
					IC50 Quinidine : 1.40 μM
MATE2-K	HEK (Transient)	SLC47A2	10 μM Metformin	KM: 252 μM	IC50 Cimetidine: 4.95 μM
			5 μM Tetraethylammonium bromide	KM: 109 μM	IC50 Cimetidine : 36.6 μM
					IC50 Quinidine : 16.3 μM
rUrat1	HEK	Slc22a12	Uric acid	KM: 677 μM	IC50 Benzbromarone : 1.88 μM
OSTα/β	HEK	SLC51	Taurocholic acid	KM: 7960 μM	IC50 Indomethacin : 262 μM

The values in parentheses show the values from references.

The chart above displays experimental conditions for Drug Transporter services as provided through our Sekisui Medical facility in Japan.

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