

ABC EFFLUX TRANSPORTER ASSAYS

ATPase Assays

Transporter	Membrane used	Reference activator substrate	Reference inhibitor
MDR1/P-gp (ABCB1)	SB-MDR1-Sf9-ATPase	Verapamil	Cyclosporin A
rat Mdr1b	SB-ratMdr1b-Sf9-ATPase	Verapamil	Cyclosporin A
MRP1 (ABCC1)	SB-MRP1-Sf9-ATPase	NEM-GS	Benzbromarone
MRP2 (ABCC2)	SB-MRP2-Sf9-ATPase	Sulfasalazine	Benzbromarone
MRP3 (ABCC3)	SB-MRP3-Sf9-ATPase	Benzbromarone	Cyclosporin A
BCRP (ABCG2)	SB-BCRP-M-ATPase	Sulfasalazine	Ko134
mouse Bsep	SB-mouseBsep-HAM-Sf9-ATPase	TCDC	Cyclosporin A

NEM-GS: N-Ethylmaleimide S-glutathione, TCDC: taurochenodeoxy-cholate, E₂17βG: estradiol-17-beta-glucuronide

Deliverables: EC₅₀, IC₅₀ • Suggested concentration range of test drug: 137 nM–300 μM (8 concentrations)

Vesicular Transport Assays

Transporter	Membrane used	Probe substrate	Positive control
MDR1/P-gp (ABCB1)	SB-MDR1-K-VT	³ H-NMQ	Verapamil
rat Mdr1b	SB-ratMdr1b-K-VT	³ H-NMQ	Verapamil
MRP1 (ABCC1)	SB-MRP1-Sf9-VT	³ H-LTC4 or radioactive form of test drug	MK571
MRP2 (ABCC2)	SB-MRP2-Sf9-VT	³ H-E ₂ 17βG or radioactive form of test drug	Benzbromarone
rat Mrp2	SB-ratMrp2-MDCKII-VT	fluorescent CDCF or radioactive form of test drug	Benzbromarone
MRP3 (ABCC3)	SB-MRP3-Sf9-VT	³ H-E ₂ 17βG or radioactive form of test drug	Benzbromarone
MRP4 (ABCC4)	SB-MRP4-LLC-PK1-VT	³ H-DHEAS (0,02 μM)	MK571
MRP5 (ABCC5)	SB-MRP5-HEK293-VT	³ H-cGMP (1 μM)	Benzbromarone
BCRP (ABCG2)	SB-BCRP-M-VT	³ H-Estrone-3-sulfate or radioactive form of test drug	Ko134
mouse Bcrp1	SB-mouseBcrp1-MDCKII-VT	³ H-Estrone-3-sulfate or radioactive form of test drug	Ko134
BSEP (ABCB11)	SB-BSEP-Sf9-VT	³ H-Taurocholate or radioactive form of test drug	Cyclosporin A
mouse Bsep	SB-mouseBsep-Sf9-VT	³ H-Taurocholate or radioactive form of test drug	Cyclosporin A

³H-NMQ: ³H-N-methyl-quinidine, ³H-LTC4: ³H-leukotriene C₄, ³H-cGMP: ³H-Guanosine 3',5'-cyclic phosphate, CDCF: 5(6)-Carboxy-2',7'-dichlorofluorescein, 3H-DHEAS: dehydroepiandrosterone-sulfate


Deliverables: IC₅₀ • Suggested concentration range of test drug: 410 nM–300 μM (7 concentrations)

Dye Transport Assays

Transporter	Cell line used	Dye (probe substrate)	Positive control
MDR1/P-gp (ABCB1)	HL60-MDR or K562-MDR	Calcein AM	Verapamil
MRP1 (ABCC1)	HL60-MRP	Calcein AM	Verapamil
BCRP (ABCG2)	MXR-M	Hoechst 33342	Ko134

Deliverables: IC₅₀ • Suggested concentration range of test drug: 0,07 μM–150 μM (8 concentrations)

UPTAKE TRANSPORTER ASSAYS

Transporter	Probe substrate	Reference inhibitor	Cell type	Negative control
OATP1B1 (SLCO1B1)/ OATP2, OATP-C	³ H-Estrone-3-Sulfate	Cerivastatin	CHO	Parental cells
OATP1B3 (SLCO1B3)/OATP8	Fluo-3	Fluvastatin	CHO	Parental cells
OATP2B1 (SLCO2B1)/OATP-B	³ H-Estrone-3-Sulfate	Fluvastatin	MDCKII	Parental cells
ratOatp1a1 (Slc1a1)	³ H-Estrone-3-Sulfate	Ketoconazole	CHO	Parental cells
NTCP (SLC10A1)	³ H-Taurocholate	TCDC	CHO	Na ⁺ free buffer
ratNtcp (Slc10a1)	³ H-Taurocholate	TCDC	CHO	Na ⁺ free buffer
PEPT1 (SLC15A1)	Gly-Sar	Tyr-Phe	CHO	Parental cells
PEPT2 (SLC15A2)	Gly-Sar	Cefadroxil	CHO	Parental cells
OAT1 (SLC22A6)	³ H-p-Aminohippuric acid (PAH)	Benzbromarone	CHO	Parental cells
OAT3 (SLC22A8)	³ H-Estrone-3-Sulfate	Probenecid	HEK293	Parental cells
OCT1 (SLC22A1)	¹⁴ C-Tetraethylammonium-chloride (TEA)	Verapamil	CHO	Parental cells
OCT2 (SLC22A2)	¹⁴ C-Tetraethylammonium-chloride (TEA)	Verapamil	CHO	Parental cells
OCT2 (SLC22A2)	¹⁴ C-Metformin	Verapamil	CHO	Parental cells
OCTN1 (SLC22A4) 	¹⁴ C-Tetraethylammonium-chloride (TEA)	Verapamil	CHO	Parental cells

Gly-Sar: Glycylsarcosine, Tyr-Phe: Tyrosine-Phenylalanine

Deliverables: IC₅₀ – for interacting compounds • (Follow-up studies are available, which provide kinetic data: Km Ki and Vmax)

MONOLAYER ASSAYS


Caco-2 Monolayer Assays

Studies	Standard setups		
	Basic Studies at one concentration, two time points	Extended Studies at several concentrations, more time points, more controls	FDA compliant Studies according to the 2006 FDA Draft Guidance
Passive permeability studies: uni- and bidirectional permeability	☑	☑	☑
Substrate assessment: Specific transporter studies	☑ P-gp ☑ BCRP	☑ P-gp ☑ BCRP	☑ P-gp
Inhibitor assessment: Drug-Drug Interaction (DDI) studies	☑	☑	☑
DDI - IC ₅₀ determination for digoxin inhibition, positive control: PSC 833		☑	☑

MDCKII-MDR1 Monolayer Assays

Studies	Standard setups		
	Basic Studies at one concentration, two time points	Extended Studies at several concentrations, more time points, more controls	FDA compliant Studies according to the 2006 FDA Draft Guidance
Substrate assessment: Specific transporter studies	☑	☑	☑
Inhibitor assessment: Drug-Drug (DDI) Interaction studies	☑	☑	☑
DDI - IC ₅₀ determination for digoxin inhibition, positive control: PSC 833		☑	☑

Additional Transfected MDCKII Monolayer Assays			
Cell line	Application	Positive control	Negative control
MDCKII- BCRP	Substrate assessment: Direct BCRP interaction, uni- and bidirectional permeability	Transport of prazosin	Parental cell line
	Inhibitor assessment: Inhibition of prazosin transport	Ko134	
MDCKII- mBcrp1	Substrate assessment: Direct mBcrp1 interaction, uni- and bidirectional permeability	Transport of prazosin	Parental cell line
	Inhibitor assessment: Inhibition of prazosin transport	Ko134	
MDCKII- OATP2B1/BCRP	Substrate assessment: Direct vectorial transport	Transport of Estrone-3-Sulfate	Parental cell line and BCRP, OATP2B1 single transfectants
	Inhibitor assessment: Inhibition of Estrone-3-Sulfate transport	Estrone and Ko134	
MDCKII- NTCP/BSEP	<i>under development</i>		
LLC-PK1- MDR1	<i>under development</i>		

Rat Brain Endothelial Cell Monolayer Assays 		
Studies	Standard setups	
	Basic Studies at one concentration	Extended Studies at several concentrations
Passive permeability studies: uni- and bidirectional permeability	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Substrate assessment: Specific transporter studies	<input checked="" type="checkbox"/> Mdr1a	<input checked="" type="checkbox"/> Mdr1a
Inhibitor assessment: Drug-Drug Interaction(DDI) studies	<input checked="" type="checkbox"/> Mdr1a	<input checked="" type="checkbox"/> Mdr1a
DDI - IC50 determination for quinidine inhibition, positive control: PSC 833	<input checked="" type="checkbox"/> Mdr1a	<input checked="" type="checkbox"/> Mdr1a

RAT BRAIN MICRODIALYSIS

In vivo **microdialysis** monitors drug distribution at peripheral tissue sites and the penetration of various agents across the blood brain barrier (BBB).

This technology is used for

- measuring unbound drug and metabolite concentrations in the interstitial space in brain and peripheral tissues including blood,
- continuous monitoring of neurotransmitter release to various stimuli,
- measuring concentrations of many analytes associated with tissue damage in CNS and in various organs.

MDR1/P-gp and BCRP are the two most important efflux transporters at the BBB. We use specific inhibitors for Mdr1 and Bcrp to determine their contribution to limit the penetration of a compound into the brain tissue.

Microdialysis assays
I. Determination of brain penetration of test molecules in rats
II. Determination of Mdr1 and Bcrp specific transporter interaction: substrate and inhibitor assessment
III. Determination of brain penetration of test molecules and simultaneous monitoring of neurotransmitter release in specified brain regions.

***IN VIVO* RAT LIVER STUDIES**

In vivo biliary efflux experiments are performed on rats. Interactions with Mrp2, Bsep and Bcrp transport proteins are determined from the altered biliary elimination of the utilized probe substrates.

GENERAL REMARKS

Results are usually available within 3 to 10 weeks. The schedule might depend on the actual workload of SOLVO's laboratory. We can provide a final schedule before contracting, including needed quantity of test drugs.

PRICES ARE AVAILABLE UPON REQUEST.