

## ABC EFFLUX TRANSPORTER ASSAYS

### ATPase Assays

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

NEM-GS: N-Ethylmaleimide S-glutathione, TCDC: taurochenodeoxy-cholate, E<sub>2</sub>17βG: estradiol-17-beta-glucuronide

Deliverables: EC<sub>50</sub>, IC<sub>50</sub> • Suggested concentration range of test drug: 137 nM–300 μM (8 concentrations)

### Vesicular Transport Assays

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

<sup>3</sup>H-NMQ: <sup>3</sup>H-N-methyl-quinidine, <sup>3</sup>H-LTC4: <sup>3</sup>H-leukotriene C<sub>4</sub>, <sup>3</sup>H-cGMP: <sup>3</sup>H-Guanosine 3',5'-cyclic phosphate, CDCF: 5(6)-Carboxy-2',7'-dichlorofluorescein, 3H-DHEAS: dehydroepiandrosterone-sulfate

Deliverables: IC<sub>50</sub> • Suggested concentration range of test drug: 410 nM–300 μM (7 concentrations)

### Dye Transport Assays

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

Deliverables: IC<sub>50</sub> • 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
<b>OATP1B1</b> (SLCO1B1)/ OATP2, OATP-C	<sup>3</sup> H-Estrone-3-Sulfate	Cerivastatin	CHO	Parental cells
<b>OATP1B3</b> (SLCO1B3)/OATP8	Fluo-3	Fluvastatin	CHO	Parental cells
<b>OATP2B1</b> (SLCO2B1)/OATP-B	<sup>3</sup> H-Estrone-3-Sulfate	Fluvastatin	MDCKII	Parental cells
<b>ratOatp1a1</b> (Slco1a1)	<sup>3</sup> H-Estrone-3-Sulfate	Ketoconazole	CHO	Parental cells
<b>NTCP</b> (SLC10A1)	<sup>3</sup> H-Taurocholate	TCDC	CHO	Na <sup>+</sup> free buffer
<b>ratNtcp</b> (Slc10a1)	<sup>3</sup> H-Taurocholate	TCDC	CHO	Na <sup>+</sup> free buffer
<b>PEPT1</b> (SLC15A1)	Gly-Sar	Tyr-Phe	CHO	Parental cells
<b>PEPT2</b> (SLC15A2)	Gly-Sar	Cefadroxil	CHO	Parental cells
<b>OAT1</b> (SLC22A6)	<sup>3</sup> H-p-Aminohippuric acid (PAH)	Benzbromarone	CHO	Parental cells
<b>OAT3</b> (SLC22A8)	<sup>3</sup> H-Estrone-3-Sulfate	Probenecid	CHO	Parental cells
<b>OCT1</b> (SLC22A1)	<sup>14</sup> C-Tetraethylammonium-chloride (TEA)	Verapamil	CHO	Parental cells
<b>OCT2</b> (SLC22A2)	<sup>14</sup> C-Tetraethylammonium-chloride (TEA)	Verapamil	CHO	Parental cells
<b>OCT2</b> (SLC22A2)	<sup>14</sup> C-Metformin	Verapamil	CHO	Parental cells

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

Deliverables: IC<sub>50</sub> – for interacting compounds • (Follow-up studies are available, which provide kinetic data: Km Ki and Vmax)

## MONOLAYER ASSAYS

### Caco-2 Monolayers

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	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Substrate assessment: Specific transporter studies	<input checked="" type="checkbox"/> P-gp <input checked="" type="checkbox"/> BCRP	<input checked="" type="checkbox"/> P-gp <input checked="" type="checkbox"/> BCRP	<input checked="" type="checkbox"/> P-gp
Inhibitor assessment: Drug-Drug Interaction studies	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
IC50 determination for digoxin inhibition, positive control: PSC 833		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

### MDCKII-MDR1 Monolayer

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	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Inhibitor assessment: Drug-Drug Interaction studies	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
IC50 determination for digoxin inhibition, positive control: PSC 833		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

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

### 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.**