SOLVO BIOTECHNOLOGY

SOLVO PREDISCREEN SERVICE CATALOGUE

ABC EFFLUX TRANSPORTER ASSAYS

ATPase Assays			
Transporter	**Membrane used**	**Probe (reference substrate)**	**Positive control activation / inhibition**
MDR1/P-gp (ABCB1)	SB-MDR1-Sf9-ATPase	Verapamil	Digoxin / Cyclosporine A
ratMdr1b	SB-ratMdr1b-Sf9-ATPase	Verapamil	Quinidine / Cyclosporine A
MRP1 (ABCC1)	SB-MRP1-Sf9-ATPase	NEM-GS	Indomethacin / Benzbromarone
MRP2 (ABCC2)	SB-MRP2-Sf9-ATPase	Probenecid	Sulfasalazine / Benzbromarone
ratMrp2	SB-ratMrp2-Sf9-ATPase	Probenecid	Sulfasalazine / Benzbromarone
MRP3 (ABCC3)	SB-MRP3-Sf9-ATPase	Benzbromarone	$E_2$17βG / Cyclosporine A
BCRP (ABCG2)	SB-BRCP-M-ATPase or SB-BCRP-HAM-Sf9-ATPase	Sulfasalazine, Hoechst 33342, Ko134	Topotecan / Ko134
BCRP-R482G (ABCG2-R482G)	SB-BCRP-R482G-Sf9-ATPase	Prazosin, Hoechst 33342, Ko134	Mitoxantrone / Ko134
mouseBsep	SB-mouseBsep-HAM-Sf9-ATPase	TCDC	

NEM-GS: N-Ethylmaleimide S-glutathione, **TCDC:** taurochenodeoxy-cholate, **$E_2$17βG:** estradiol-17-beta-glucuronide

Deliverables: EC_{50}, IC_{50} • Suggested concentration range of test drug: 137 nM–300 µM (8 concentrations)

Vesicular Transport Assays			
Transporter	**Membrane used**	**Probe (reference substrate)**	**Positive control**
MDR1/P-gp (ABCB1)	SB-MDR1-K-VT	^{3}H-NMQ	Verapamil
ratMdr1b	SB-ratMdr1b-K-VT	^{3}H-NMQ	Verapamil
MRP1 (ABCC1)	SB-MRP1-Sf9-VT	^{3}H-LTC4 or radioactive form of test drug (TD)	MK571
MRP2 (ABCC2)	SB-MRP2-Sf9-VT	^{3}H-$E_2$17βG or radioactive form of TD	Benzbromarone
ratMrp2	SB-ratMrp2-MDCKII-VT	fluorescent CDCF	Benzbromarone
MRP3 (ABCC3)	SB-MRP3-Sf9-VT	^{3}H-$E_2$17βG or radioactive form of TD	Benzbromarone
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 TD	Methotrexate
BCRP (ABCG2)	SB-BCRP-HAM-Sf9-VT	^{3}H-Estrone-3-sulfate or 3H-Methotrexate	Methotrexate
BCRP (ABCG2)	SB-BCRP-Sf9-VT	^{3}H-Methotrexate or radioactive form of TD	Estrone-3-sulfate
BSEP (ABCB11)	SB-BSEP-Sf9-VT	^{3}H-Taurocholate or radioactive form of TD	Cyclosporine A
mouseBsep	SB-mouseBsep-Sf9-VT	^{3}H-Taurocholate	Cyclosporine A

^{3}H-NMQ: ^{3}H-N-methyl-quinidine, **^{3}H-LTC4:** ^{3}H-leukotriene C4, **^{3}H-cGMP:** ^{3}H- Guanosine 3′,5′-cyclic phophate, **CDCF:** 5(6)-Carboxy-2′,7′-dichlorofluorescein

Deliverables: IC_{50} – for interacting compounds • (Follow-up studies are available, which provide kinetic data: K_m K_i and V_{max})
Suggested concentration range of test drug: 0,41 µM–300 µM (7 concentrations)

Dye Transport Assays			
Transporter	**Cell line used**	**Dye used**	**Positive control**
MDR1/P-gp (ABCB1)	HL60-MDR or K562-MDR	Calcein AM	Verapamil
MRP1 (ABCC1)	HL60-MRP	Calcein AM	MK571
BCRP (ABCG2)	MXR-M	Hoechst 33342	Proprietary

Deliverables: IC_{50} • Suggested concentration range of test drug: 0,07 µM–150 µM (8 concentrations)

UPTAKE TRANSPORTER ASSAYS

Transporter	Probe (reference substrate)	Reference inhibitor	Cell type	Negative control
OATP1B1 (SLCO1B1)	^{3}H-Estrone-3-Sulfate	Cerivastatin	CHO	Parental cells
OATP1B3 (SLCO1B3)	Fluo-3	Fluvastatin	CHO	Parental cells
OATP2B1 (SLCO2B1)	^{3}H-Estrone-3-Sulfate	Fluvastatin	MDCKII	Parental cells
ratOatp1a1 (Slco1a1)	^{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	^{3}H-p-Aminohippuric acid (PAH)	Benzbromarone	CHO	Parental cells
OCT1	^{14}C-Tetraethylammonium-chloride (TEA)	Verapamil	CHO	Parental cells
OCT2	^{14}C-Tetraethylammonium-chloride (TEA)	Verapamil	CHO	Parental cells

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

Deliverables: IC_{50} – for interacting compounds • (Follow-up studies are available, which provide kinetic data: K_m K_i and V_{max})
Suggested concentration range of test drug: 0,41 µM–300 µM (7 concentrations)

MONOLAYER ASSAYS

Caco-2 Monolayers						
Studies	Standard setups					
	Basic Studies at one concentration		Extended Studies at several concentrations, more controls		FDA Guidelines Studies according to FDA draft guidance (2006)	
Passive permeability studies	✔		✔		✔	
Specific transporter studies	✔ P-gp	✔ BCRP	✔ P-gp	✔ BCRP	✔ P-gp	
Drug-Drug Interaction studies	✔		✔		✔	
IC_{50} determination for digoxin inhibition	✔		–		✔	

MDCKII Monolayers			
Cell line	Application	Positive control	Negative control
MDCKII-*BCRP*	Direct BCRP interaction	Transport of prazosin	Parental cell line
	Inhibition of prazosin transport	Ko134	NA
MDCKII-*mBcrp1*	Direct mBcrp1 interaction	Transport of prazosin	Parental cell line
	Inhibition of prazosin transport	Ko134	NA
MDCKII-*OATP2B1/BCRP*	Direct vectorial transport	Estrone-3-Sulfate transport	Parental cell line and BCRP, OATP2B1 single transfectants
	Inhibition of Estrone-3-Sulfate transport	Estrone and Ko134	NA
MDCKII-*MDR1*	Direct MDR1 interaction	Transport of digoxin	Parental cell line
	Inhibition of digoxin transport	Verapamil	NA
MDCKII-*NTCP/BSEP*	*under development*		
LLC-PK1-*MDR1*	*under development*		

B-CLEAR® SERVICES

B-CLEAR® assays – rat	B-CLEAR® assays – human
Mrp2 efflux inhibition with Estradiol-17-beta-glucuronide as probe substrate	*MRP2* efflux inhibition with Estradiol-17-beta-glucuronide as probe substrate – *under development*
Mrp2 efflux inhibition with Bilirubin glucuronide as probe substrate	*MRP2* efflux inhibition with Bilirubin glucuronide as probe substrate
Bsep efflux inhibition with Taurocholate as probe substrate	*BSEP* efflux inhibition with Taurocholate as probe substrate – *under development*
Direct efflux measurements	Direct efflux measurements
Induction experiments – *under development*	Induction experiments – *under development*

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 test molecule interactions with transporters: substrate / inhibitor
III. Determination of brain penetration of test molecules and simultaneous monitoring of neurotransmitter release in specified brain regions.

IN VIVO ANIMAL 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

Screening 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.

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