

## I. PRODUCTS

### 1. PREDEASY™ ATPase Assay Kits

Product name	Intended use	Reference substrate
SB-MDR1/P-gp- <b>PREDEASY™</b> ATPase	ATPase activation and inhibition	Verapamil
SB-ratMdr1b- <b>PREDEASY™</b> ATPase	ATPase activation and inhibition	Verapamil
SB-MRP1- <b>PREDEASY™</b> ATPase	ATPase activation and inhibition	NEM-GS
SB-MRP2- <b>PREDEASY™</b> ATPase	ATPase activation and inhibition	Sulfasalazine
SB-MRP3- <b>PREDEASY™</b> ATPase	ATPase activation and inhibition	Benzbromarone
SB-BCRP-HAM- <b>PREDEASY™</b> ATPase	ATPase activation and inhibition	Sulfasalazine
SB-mouseBsep-HAM- <b>PREDEASY™</b> ATPase	ATPase activation and inhibition	Taurochenodeoxycholate
SB-defBCRP-HAM- <b>PREDEASY™</b> -CTRL	ATPase activation and inhibition	-
SB-defMRP- <b>PREDEASY™</b> CTRL	ATPase activation and inhibition	-
SB-Beta-gal- <b>PREDEASY™</b> CTRL	ATPase activation and inhibition	-

**PREDEASY™ ATPase Assay Kits** are available in three sizes -1, 3 and 10 (96 well) **plate sizes**. All reagents and solutions are in one easy-to-use kit, and accompanied by a CD that includes the assay protocol, data sheet, MSDS, electronic data processing and evaluation templates and example data. The recommended layout allows activation and inhibition data in duplicate for 8 concentrations of the test article with all of the relevant controls. These kits deliver many advantages when compared to conventional ATPase products.

### 2. PREDIVEZ™ Vesicular Transport Assay Kits

Product name	Intended use	Reference substrate
SB-MDR1/P-gp- <b>PREDIVEZ™</b> -VT kit	Vesicular transport	<sup>3</sup> H-N-methyl-quinidine
SB-ratMdr1b- <b>PREDIVEZ™</b> -VT kit	Vesicular transport	<sup>3</sup> H-N-methyl-quinidine
SB-MRP2- <b>PREDIVEZ™</b> -VT kit	Vesicular transport	fluorescent CDCF
SB-MRP3- <b>PREDIVEZ™</b> -VT kit	Vesicular transport	fluorescent CDCF
SB-MRP5- <b>PREDIVEZ™</b> -VT kit	Vesicular transport	fluorescent CDCF
SB-BCRP-M- <b>PREDIVEZ™</b> -VT kit	Vesicular transport	fluorescent Lucifer Yellow

PREDIVEZ vesicular transport kits are available in three sizes 3, 6 and 9 compound sizes.

Kits contain sufficient amount of reagents and membrane preparation for testing 3 compounds per 96-well plate. Each kit is accompanied by a CD that includes the assay protocol, Excel spreadsheet with data processing template, MSDS and reference curves with known interactors. In case of MDR1 and ratMdr1b kits the labeled reference substrate is shipped separately.

### 3. B-CLEAR® Kits

Product name
B-CLEAR® Kit – rat
B-CLEAR® Kit – human Under development

**B-CLEAR®** sandwich-cultured hepatocyte system is an in vitro method to predict hepatic uptake, biliary excretion and assess drug transporter inhibition. SOLVO is the exclusive distributor of B-CLEAR® Kits in Europe.

## 4. MEMBRANE PRODUCTS

### A. MEMBRANE PREPARATIONS FOR ATPASE ASSAYS

Transporter (gene)	Catalogue #	Intended use	Reference substrate	Membrane <sup>1</sup> use per assay well	Basal ATPase activity <sup>2</sup> [nmol Pi/mg/min]	Modified ATPase activity <sup>2,3</sup> [nmol Pi/mg/min]	-fold activation
<b>MDR1 / P-gp (ABCB1)</b>	SB-MDR1-Sf9-ATPase	ATPase activation ATPase inhibition	Verapamil (40 µM) Verapamil (40 µM)	40 µg	8-22	30-90	3-5
<b>rat Mdr1b</b>	SB-ratMdr1b-Sf9-ATPase	ATPase activation ATPase inhibition	Verapamil (200 µM) Verapamil (200 µM)	20 µg	10-25	70-100	4-8
<b>MRP1 (ABCC1)</b>	SB-MRP1-Sf9-ATPase	ATPase activation ATPase inhibition	NEM-GS (10 mM) NEM-GS (10 mM)	40 µg	5-10	10-25	2-3
<b>MRP2 (ABCC2)</b>	SB-MRP2-Sf9-ATPase	ATPase activation ATPase inhibition	Probenecid (1 mM) Probenecid (1 mM)	20 µg	6-12	25-40	2.5-4
<b>rat Mrp2</b>	SB-ratMrp2-Sf9-ATPase	ATPase activation ATPase inhibition	Probenecid (4 mM) Probenecid (4 mM)	20 µg	5-10	12-30	2-4
<b>MRP3 (ABCC3)</b>	SB-MRP3-Sf9-ATPase	ATPase activation ATPase inhibition	Benzbromarone (50 µM) Benzbromarone (50 µM)	20 µg	6-13	18-30	2-4
<b>BCRP / MXR (ABCG2)</b>	SB-BCRP-HAM-Sf9-ATPase	ATPase activation ATPase inhibition	Sulfasalazine (10 µM) Sulfasalazine (10 µM)	20 µg	5-15	15-40 0-3	2-4 0-1
<b>BCRP / MXR (ABCG2)</b>	SB-BCRP-M-ATPase	ATPase activation ATPase inhibition	Sulfasalazine (10 µM) Hoecht 33342 (10 µM) / KO143 (100 nM)	20 µg	5-15	15-40 0-3	2-4 0-1
<b>BCRP / MXR -R482G (ABCG2-R482G)<sup>4</sup></b>	SB-BCRP-R482G-Sf9-ATPase	ATPase activation ATPase inhibition	Prazosine (50 µM) Hoecht 33342 (10 µM) / KO143 (100 nM)	20 µg	30-80	50-150 5-25	1.5-2 0-1
<b>defMRP</b>	SB-defMRP-Sf9-CTRL	Control for MRP1-, MRP2-, ratMrp2-, MRP3-Sf9 ATPase assay	See above MRPs and ratMrp2 reference substrates	20 or 40 µg	5-15	5-15 (No activation)	-
<b>defBCRP</b>	SB-defBCRP-Sf9-CTRL	Control for BCRP-R482G-Sf9 ATPase assay	See above SB-BCRP-R482G Sf9 reference substrates	20 or 40 µg	0-10	0-10 (No activation)	-
<b>M</b>	SB-M-CTRL	Control for BCRP-M ATPase assay	See above SB-BCRP-M reference substrates	20 µg	0-5	0-5 (No activation)	-
<b>Beta-gal</b>	SB-beta-gal-Sf9-CTRL	Control for MDR1, ratMdr1b, MRP1-, MRP2-, ratMrp2-, MRP3-Sf9 ATPase assay	See above MDR1, MRPs, ratMdr1b and ratMrp2 reference substrates	20 or 40 µg	5-15	5-15 (No activation)	-

Sf9=Spodoptera frugiperda ovarian; M=Mammalian, HAM = High Activity Membrane

## B. MEMBRANE PREPARATIONS FOR VESICULAR TRANSPORT ASSAYS

Transporter (gene)	Catalogue #	Intended use	Reference substrate	Membrane <sup>1</sup> use per assay well	ATP dependent transport [pmol /mg/min]
<b>MDR1/P-gp (ABCB1)</b>	SB-MDR1-K-VT	Vesicular transport	3H-NMQ	50 µg	350-500
<b>ratMdr1b</b>	SB-ratMdr1b-Sf9-VT	Vesicular transport	3H-NMQ	50 µg	30-80
<b>MRP1 (ABCC1)</b>	SB-MRP1-Sf9-VT	Vesicular transport	<sup>3</sup> H-LTC <sub>4</sub> (50 nM)	50 µg	20-45
<b>MRP2 (ABCC2)</b>	SB-MRP2-Sf9-VT	Vesicular transport Vesicular transport	<sup>3</sup> H-E <sub>2</sub> -17βG (50 µM) <sup>3</sup> H-LTC <sub>4</sub> (50 nM)	50 µg	300-800 6-20
<b>MRP3 (ABCC3)</b>	SB-MRP3-Sf9-VT	Vesicular transport	<sup>3</sup> H-E <sub>2</sub> -17βG (1 µM)	50 µg	20-35
<b>MRP4 (ABCC4)</b>	Under development				
<b>MRP5 (ABCC5)</b>	SB-MRP5-HEK293-VT	Vesicular transport	<sup>3</sup> H-cGMP (1 µM)	30 µg	0,4-0,7
<b>BCRP (ABCG2)</b>	SB-BCRP-HAM-Sf9-VT	Vesicular transport	<sup>3</sup> H-Estrone-3-sulfate (1 µM) <sup>3</sup> H-Methotrexate (100 µM)	25 µg 50 µg	80-160 150-250
<b>BCRP (ABCG2)</b>	SB-BCRP-M-VT	Vesicular transport Vesicular transport	<sup>3</sup> H-Estrone-3-sulfate (1 µM) <sup>3</sup> H-Methotrexate (100 µM)	25 µg 50 µg	40-100 40-90
<b>mouse Bcrp</b>	Under development				
<b>BSEP (ABCB11)</b>	SB-BSEP-Sf9-VT	Vesicular transport	<sup>3</sup> H-Taurocholate (2 µM)	50 µg	15-35
<b>mouse Bsep</b>	SB-mouseBsep-Sf9-VT	Vesicular transport	<sup>3</sup> H-Taurocholate (2 µM)	50 µg	100-400
<b>rat Bsep</b>	Under development				
<b>defMRP</b>	SB-defMRP-Sf9-CTRL	Control for MRP1-, MRP2-, MRP3-, BSEP- and Vesicular transport assay	<sup>3</sup> H-LTC <sub>4</sub> (50 nM) <sup>3</sup> H-E <sub>2</sub> -17βG (50 µM) <sup>3</sup> H-E <sub>2</sub> -17βG (1 µM)	50 µg	<6 <50 <2
<b>defBCRP-HAM</b>	SB-defBCRP-HAM-Sf9-CTRL	Control for BCRP-HAM Vesicular transport assay	<sup>3</sup> H-Estrone-3-sulfate <sup>3</sup> H-Methotrexate	25 µg 50 µg	<5 <5
<b>M</b>	SB-M-CTRL	Control for BCRP-M Vesicular transport assay	<sup>3</sup> H-Estrone-3-sulfate (1 µM) <sup>3</sup> H-Methotrexate (100 µM)	25 µg 50 µg	<5 <5
<b>HEK293</b>	SB-HEK293-CTRL	Control for MRP5 Vesicular transport assay	<sup>3</sup> H-cGMP (1 µM)	30 µg	0,05-0,15
<b>beta-gal</b>	SB-beta-gal-CTRL	Control for MRP1-, MRP2-, MRP3-, BSEP- and mouseBsep Vesicular transport assay	<sup>3</sup> H-LTC <sub>4</sub> (50 nM) <sup>3</sup> H-E <sub>2</sub> -17βG (50 µM) <sup>3</sup> H-E <sub>2</sub> -17βG (1 µM) <sup>3</sup> H-Taurocholate (2 µM)	50 µg	<6 <50 <2 <5

(1) Recommended values. These parameters can be modified according to one's actual needs.

(2) Vanadate-sensitive

(3) In the presence of the reference substrate

(4) Mutant version (R482G)

## II. PREDISCREEN SERVICES

### 1. ABC (EFFLUX) TRANSPORTER ASSAYS

#### A. ATPase ASSAYS [ATPase ACTIVATION + INHIBITION]

Transporter	Membrane used	Reference substrate	Positive control activation / inhibition
MDR1 / P-gp (ABCB1)	SB-MDR1-Sf9-ATPase	Verapamil	Digoxin / Cyclosporine A
rat Mdr1b	SB-ratMdr1b-Sf9-ATPase	Verapamil	Quinidine / Cyclosporine A
MRP1 (ABCC1)	SB-MRP1-Sf9-ATPase	NEM-GS	Indomethacin / Benzbromarone
MRP2 (ABCC2)	SB-MRP2-Sf9-ATPase	Sulfasalazine	Sulfasalazine / Benzbromarone
rat Mrp2	SB-ratMrp2-Sf9-ATPase	Probenecid	Sulfasalazine / Benzbromarone
MRP3 (ABCC3)	SB-MRP3-Sf9-ATPase	Benzbromarone	E217βG / Cyclosporine A
BCRP (ABCG2)	SB-BCRP-M-ATPase or SB-BCRP-HAM-Sf9-ATPase	Sulfasalazine, Hoechst 33342, K0143	Topotecan / K0143
BCRP – R482G <sup>1</sup> (ABCG2-R482G)	SB-BCRP-R482G-Sf9-ATPase	Prazosin, Hoechst 33342, K0143	Mitoxantron / K0143

Substrate / inhibitor used: test drug or test drug + posCTRL

Deliverables: EC<sub>50</sub>, IC<sub>50</sub>

Suggested concentration range of test drug: 137 nM – 300 μM

(1) Mutant variant (not wild type)

#### B. VESICULAR TRANSPORT ASSAYS

Transporter	Membrane used	Reference (reporter) substrate used	Positive control
MDR1/P-gp (ABCB1)	SB-MDR1-K-VT	<sup>3</sup> H-N-methyl-quinidine	Verapamil
ratMdr1b	SB-ratMdr1b-K-VT	<sup>3</sup> H-N-methyl-quinidine	Verapamil
MRP1 (ABCC1)	SB-MRP1-Sf9-VT	<sup>3</sup> H-LTC <sub>4</sub> or radioactive form of test drug (TD)	MK571
MRP2 (ABCC2)	SB-MRP2-Sf9-VT	<sup>3</sup> H-E <sub>2</sub> 17βG or radioactive form of TD	Benzbromarone
MRP3 (ABCC3)	SB-MRP3-Sf9-VT	<sup>3</sup> H-E <sub>2</sub> 17βG or radioactive form of TD	Benzbromarone
MRP4 (ABCC4)	Under development		
MRP5 (ABCC5)	SB-MRP5-HEK293-VT	<sup>3</sup> H-cGMP	Quercetin, Benzbromarone
BCRP (ABCG2)	SB-BCRP-M-VT	<sup>3</sup> H-Estrone-3-sulfate or radioactive form of TD	Methotrexate
BCRP (ABCG2)	SB-BCRP-HAM Sf9-VT	<sup>3</sup> H-Estrone-3-sulfate	Methotrexate
mouse Bcrp	Under development		
BSEP (ABCB11)	SB-BSEP-Sf9-VT	<sup>3</sup> H-Taurocholate or radioactive form of TD	Cyclosporin A
mouse Bsep	SB-MouseBsep-Sf9-VT	<sup>3</sup> H-Taurocholate or radioactive form of TD	Cyclosporin A
rat Bsep	Under development		

Deliverables: IC<sub>50</sub> – for interacting compounds

(Follow-up studies are available, which provide kinetic data: K<sub>m</sub>, K<sub>i</sub> and V<sub>max</sub>)

Concentration range of test drug: 0.41 μM – 300 μM

#### C. DYE TRANSPORT ASSAYS

Transporter	Cell line used	Dye used
MDR1 / P-gp (ABCB1)	HL60-MDR or K562-MDR	Calcein AM
MRP1 (ABCC1)	HL60-MRP	Calcein AM
BCRP (ABCG2)	BCRP-M	Hoechst 33342

Deliverables: IC<sub>50</sub>

Suggested concentration range of test drug: 0,07 μM – 150 μM

## 1. UPTAKE TRANSPORTER ASSAYS

Transporter	Reference (reporter) substrate used
NTCP (SLC10A1)	<sup>3</sup> H-Taurocholate
rat Ntcp	<sup>3</sup> H-Taurocholate
OATP-C /OATP2 (SLC01B1)	<sup>3</sup> H-Estrone-3-sulfate
OATP8 / OATP1B3 (SLC01B3)	Fluo3
OATP-B / OATP2B1 (SLC02B1)	<sup>3</sup> H-Estrone-3-sulfate
rat Oatp1 (Oatp1a1)	<sup>3</sup> H-Estrone-3-sulfate
PEPT1 (SLC15A1)	<sup>3</sup> H-Gly-Sar
PEPT2 (SLC15A2)	<sup>3</sup> H-Gly-Sar
OAT	<sup>3</sup> H-p-Aminohippuric acid (PAH)
OCT1	<sup>14</sup> C-Tetraethylammonium-chloride (TEA)

## 2. MONOLAYER ASSAYS

Barrier models			
Barrier model	Cell type	Assay type	Reference compounds
intestine	Caco-2	AB permeability, sink conditions	Antipyrin, Lucifer Yellow
kidney	MDCKII	AB and/or BA	Antipyrin, Lucifer Yellow
kidney	LLC-PK	AB and/or BA	Antipyrin, Lucifer Yellow

Specific transporter studies			
Barrier model	Cell type	Assay type	Reference compounds
P-gp	Caco-2	AB/BA permeability (efflux ratio)	Digoxin
P-gp	Caco-2	AB and/or BA with and without inhibitors	Digoxin, Inhibitor
P-gp	Caco-2	Drug-Drug-Interaction	Digoxin
BCRP	MDCKII parental and BCRP expressing	AB and/or BA	Prazosin
OATP-B and BCRP	MDCKII parental, single and double transfected	AB and BA	<sup>3</sup> H-Estrone-3-sulfate
mouseBcrp1	MDCKII parental and mouseBcrp expressing	AB and/or BA	Prazosin
OATPs and MRP2		Under development	
NTCP and BSEP		Under development	

## 3. B-CLEAR® system

B-CLEAR® assay - rat	B-CLEAR® assay - human
Mrp2 efflux inhibition with Estradiol-17-beta-glucuronide	MRP2 efflux inhibition with Estradiol-17-beta-glucuronide - under development
Mrp2 efflux inhibition with Bilirubin glucuronide	MRP2 efflux inhibition with Bilirubin glucuronide
Bsep efflux inhibition with Taurocholate	BSEP efflux inhibition with Taurocholate - under development
Direct efflux measurements	Direct efflux measurements
Induction experiments - under development	Induction experiments- under development

## 4. IN VIVO ASSAYS

Assay
Brain microdialysis studies in rats – available from Q2 2008
In vivo rat experiments

## GENERAL REMARKS

SCREENING RESULTS ARE USUALLY AVAILABLE WITHIN 4 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.  
SIGNIFICANT DISCOUNTS ARE AVAILABLE FOR LONG TERM CONTRACTS!  
ADDITIONAL VOLUME BASED DISCOUNTS ARE ALSO AVAILABLE!



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