SOLVO®
BIOTECHNOLOGY
A CHARLES RIVER COMPANY

MEMBRANE TRANSPORTER PRODUCTS

1.

MEMBRANE TRANSPORTER SERVICES

SOLVO's transporter assays are available in predefined assay setups and are also fully tailorable for the specific needs of any project, including screening-type studies. For most transporter assays, multiple probe substrate and reference inhibition options are available ensuring flexibility in assay design.

Regulatory study packages are available for in vitro DDI studies, covering transporters selected based on guidance recommendations.

Efflux Transporter Assays

Efflux (or ABC) transporters are mostly investigated for their role in limiting drug permeability to certain tissues and contributing to their active removal from certain cells.

Compound interaction with efflux transporters can be studied in 2 different assay systems. Selection of the appropriate system, assay setup and controls included for assessing a specific test article as transport substrate or inhibitor, is driven by compound- and project-specific parameters.

Monolayer Assays

Monolayer Assays are performed using polarized cell monolayers overexpressing the efflux transporter of interest via stable transfection (in an MDCKII or LLC-PK1 cellular background*).

Vesicular Transport Assays

The Vesicular Transport (VT) Assays utilize inside-out membrane vesicles generated from cells overexpressing the transporter of interest.

Human Efflux Transporters

Transporter	Available assay systems**
BCRP	Monolayer assay, Vesicular Transport assay
MDR1 (P-gp)	
BSEP	Vesicular Transport assay
MRP1	
MRP2	
MRP3 (ABCC3)	
MRP4 (ABCC4)	
MRP5 (ABCC5)	

Preclinical Species Efflux Transporters

Transporter	Species	Available assay systems
Mdr1 or Mdr1a	Mouse	Monolayer assay, Vesicular Transport assay
	Rat	Monolayer assay
	NHP (cynomolgus monkey)	Vesicular Transport assay
Bcrp	Mouse	Monolayer assay, Vesicular Transport assay
	Rat	
Bsep	NHP (cynomolgus monkey)	
	Mouse	
	Rat	
Mrp2	NHP (cynomolgus monkey)	Vesicular Transport assay
	Dog	
	Rat	

*Certain transporters available in other cellular backgrounds as well, for further information, please contact us at solvobiotech@crl.com.

Uptake Transporter Assays

Uptake (or SLC) transporters, depending on their location, can contribute both to drug absorption and elimination processes. Beyond their inclusion in drug-drug interaction and organ toxicity prediction studies, uptake transporters may also be leveraged for tissue-specific drug delivery or constitute therapeutic targets.

Compound interaction with uptake transporters (substrate or inhibition) is studied using stably transfected transporter-overexpressing cell lines in a plated format (using a HEK293 cellular background**).

Human Uptake Transporters

ASBT	MCT8	OATP1B3	PEPT2
CNT1	MCT10	OATP1A2	SGLT1
CNT2	MDR1	OATP2A1	SGLT2
CNT3	NIS	OATP2B1	SGLT5
ENT1	NTCP	OCT1	SGLT6
ENT2	OAT1	OCT2	THR1
ENT4	OAT2	OCT3	THR2
HPT1	OAT3	OCTN1	URAT1
LAT1	OAT4	OCTN2	
MATE1	OAT7	OSTα/β	
MATE2-K	OATP1B1	PEPT1	

Preclinical Species Uptake Transporters

Mouse	Rat	NHP	Dog
mouseSglt2	ratAsbt	cyNtcp	dogSglt2
	ratNtcp	cyOatp1b1	
	ratOat1	cyOatp1b3	
	ratOatp1a1	cyOatp2b1	
	ratOatp1a4		
	ratOatp1b2		
	ratOctn2		
	ratOst α/β		

*Certain transporters available in other cellular backgrounds as well, for further information, please contact us at

Multi-transporter monolayer assays

To better approximate physiological barriers, polarized cell monolayers expressing multiple transporters (efflux and/or uptake) can also be used. These cells express transporters either intrinsically (e.g., Caco-2) or via stable transfection (in MDCKII cellular background). Additionally, transporter knockout (KO) Caco-2 cells allow for determining the potential impact of specific transporters in drug absorption and disposition without dependence on chemical inhibitors.

Human Assay Systems

Caco-2 Monolayer Assays

Transporters	Cell line	Barrier modeled
BCRP	Caco-2 or C2BBe1-BCRP-KO	Intestinal, BBB, general permeability
MDR1	Caco-2 or C2BBe1-MDR1-KO	
MRP2	Caco-2 or C2BBe1-MRP2-KO	

Double Transfected Monolayer Assays

Transporters	Cell line	Barrier modeled
OATP2B1/BCRP	Transfected MDCKII	Placenta
OAT1/BCRP	Transfected MDCKII	Kidney
OAT3/BCRP	Transfected MDCKII	
OCT2/MATE1	Transfected MDCKII	
OCT2/MATE2-K	Transfected MDCKII	

MDCKII Monolayer Assays

Transporters	Cell line	Barrier modeled
-	MDCKII-Abcb1-KO	
-	Parental MDCKII	general permeability

Custom assay development

For transporters interaction assessment and related ADME-Tox services beyond our standard portfolio, custom assay development is also offered as tailored service, carried out by our RnD team. For transporter interactions, fully characterized and validated assays can be developed using stable transfection in the cell line of choice, which allows for reliable quantitative measurements and investigative studies. Alternatively, for screening-type and earlier phase studies, transient transporter expressing systems can be generated for qualitative interaction assessment. Depending on the project needs, alternative approaches can also be applied.

2.

METABOLISM SERVICES

To complement the transporter portfolio SOLVO also offers in vitro assays for the evaluation of metabolism-related drug interactions, estimation of hepatic clearance, and metabolite identification (Met ID). CYP-interaction assessment assays have been developed to meet regulatory requirements for in vitro DDI submission. In addition to these standard setups, flexible assay design and screening-type approaches are also available for our metabolism services.

CYP inhibition assays

CYP inhibition assays are conducted in a setup that allows assessment of reversible or time-dependent inhibition of CYP function. This is achieved via preincubation of the tested compound at multiple concentrations in microsomes with and without NADPH. The assay is performed using selective probe substrates to specifically test for inhibition of several P450 isoforms.

Service*	System	Species	Enzymes	Endpoints
CYP inhibition	Pooled human liver microsomes	Human	CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4	% inhibition, IC_{50} and IC_{50} shift, K_i, K_{inact}
	Pooled rat liver microsomes	Rat	Cyp1a1, Cyp2b1, Cyp2c11, Cyp2d1, Cyp3a1	

* SOLVO strives to offer highly characterized assays and therefore develops continuously. Please discuss your need for other species or assay types with our business development team who give you latest options available.

CYP induction assays

Sandwich-cultured hepatocyte assays are available for the investigation of the potential of an investigational drug to act as an inducer of major CYP enzymes. Induction potential is evaluated by examining the fold-change in CYP enzyme mRNA level (measured by RT-qPCR), and/or in CYP activity (measured by LC-MS/MS) using a probe substrate.

Service*	System	Species	Enzymes	Endpoints
CYP induction	Cryopreserved plateable human hepatocytes	Human	CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4	Fold-change in mRNA level Fold-change in CYP activity, TA recovery, Cytotoxicity assessment (LDH)
	Cryopreserved plateable rat hepatocytes	Rat	Cyp1a1, Cyp2b1, Cyp2c11, Cyp3a1	

*Number of donors and end points used are selected specifically for each project.

Metabolic stability assays

In vitro metabolic stability studies at SOLVO are available using human assay systems, or various species. For determining the in vitro half-life (min) and the in vitro clearance (µL/min per mg protein or per million cells) of the compound, its presence in assay system is monitored over time, and controls are included for distinction of metabolism-dependent loss from substrate. Compound half-life and intrinsic clearance is calculated for use in in vivo clearance predictions.

Service	System	Species	Enzymes	Endpoints
Metabolic stability test	Cryopreserved suspension hepatocytes, pooled liver microsomes or S9 fraction	Human, cynomolgus monkey, dog, rat, mouse	various	Intrinsic clearance, half-life

Metabolite identification

Metabolite identification is conducted in the same assay system as metabolic stability studies, but uses higher concentrations of the test drug and increased amount of enzyme sources (hepatocytes or subcellular fractions) to ensure detectable levels of metabolites*. Structures are proposed for putative metabolites based on the accurate mass measurement and any mass spectrometric fragmentation pattern obtained compared to that of the parent compound.

Service*	System	Species	Enzymes	Endpoints
Metabolite identification	Cryopreserved suspension hepatocytes, pooled liver microsomes or S9 fraction	Human, cynomolgus monkey, dog, rat, mouse	various	Structures proposed for putative metabolites

*For a screening type of approach, samples generated in the metabolic stability assay may be analyzed for metabolic identification as well

3.

CELLULAR UPTAKE TRANSPORTER SYSTEMS

Hepatocyte uptake assays

Hepatocyte Uptake assays utilize cryopreserved primary hepatocytes to investigate temperature-, time- and concentration-dependent intracellular accumulation of small molecules. Interactions with transporters expressed in hepatocytes (such as OATP1B1, OATP1B3, OCT1, OAT2, etc.) can also be tested. The assay also allows for Km/Vmax determination as well as drug-drug interaction assessment. This assay is offered using plated hepatocytes (upon request, oil-spin method-based approach is also available).

Sandwich-Cultured Hepatocyte Services

Sandwich cultured hepatocyte (SCH) assays utilize primary hepatocytes grown between layers of collagen, which allows retention of physiological morphology and function, including the formation of functional canalicular bile pockets (analogous to bile canaliculi in vivo). Using patented B-CLEAR®* technology, biliary efflux and clearance, and biliary transporter interactions can be determined.

HepatoPac® Micropatterned Hepatocyte Co-cultures*

HepatoPac® utilizes micropatterned co-cultures (MPCCs) of primary hepatocytes and stromal cells for studying hepatic transport, metabolism, and toxicity in vitro. Simulating the micro-scale in vivo architecture of the liver allows the retention of physiological transporter and metabolic enzyme expression and activity over several weeks in culture. Potential applications include xeno- and endobiotic transport studies, metabolite identification and analysis, induction studies, and mechanistic and predictive toxicology studies, with long-term multiple dosing.

Renal Proximal Tubule Cell Monolayer Services

The Renal Proximal Tubule Cell (PTC) monolayer model uses freshly isolated primary human or preclinical animal PTCs for investigating renal drug handling. Physiological expression and function of transporters, metabolizing enzymes, and signaling proteins is retained, making the assay suitable for many applications, including transport assays, receptor-mediated endocytosis, investigation of signaling pathways, cross-species differences, and predictive or mechanistic toxicity studies utilizing clinically relevant biomarkers.
PTC assays are offered in partnership with Newcells Biotech, Newcastle, UK.

Rodent Brain Endothelial Cell (BEC) Monolayer Assays
The Brain Endothelial Cell monolayer assays using rat- or mouse cells (RBEC or MBEC) studies the brain penetration of compounds by evaluating their vectorial transport across an endothelial cell monolayer, analogous to in vivo brain capillaries. This system is a co-culture of three primary cell types: endothelial cells, pericytes and astrocytes, which ensures barrier formation and expression of key enzymes, including transporters. The BEC monolayer assay is also a great tool for predicting or corroborating in vivo brain permeability (Kp,uu) data.
R/MBEC assays are offered in partnership with the Biological Research Center of Szeged, Hungary.

BCS-based Permeability Classification for Biowaiver (Caco-2 model)
SOLVO's Caco-2 package for permeability classification, fully validated in accordance with the ICH M9 guidance, is used to provide the definite permeability class of a compound in line with the BCS classification system, which is also essential for biowaiver submissions.
In vitro permeability of a test article (TA) is determined based on comparison to the high-low permeability boundary cut-off compound (Minoxidil) in a highly regulated assay. The BCS Biowaiver Caco-2 package contains a defined series of supporting assays and applies a stepwise approach to ascertain all regulatory requirements are met.

Service	Species availability
Hepatocyte Uptake Assays – Plated format	Human, Mouse, Rat, NHP, Dog
Hepatocyte Uptake Assays – Oil-spin method	Human, Mouse, Rat, NHP, Dog
Sandwich-Cultured Hepatocyte (SCH) Assays	Human, Rat
HepatoPac MetID – long term incubation	Human, Rat, Dog
HepatoPac Metabolic Stability – long term incubation	Human, Rat
HepatoPac Toxicity – long term incubation	
Renal Proximal Tubule Cell (PTC) Monolayer Services	Human, Rat, NHP
Rodent Brain Endothelial Cell (BEC) Monolayer Assays	Rat, Mouse, Porcine*
BCS-based Permeability Classification (Caco-2 model)	Human

*Additional species may be available upon request.

4.

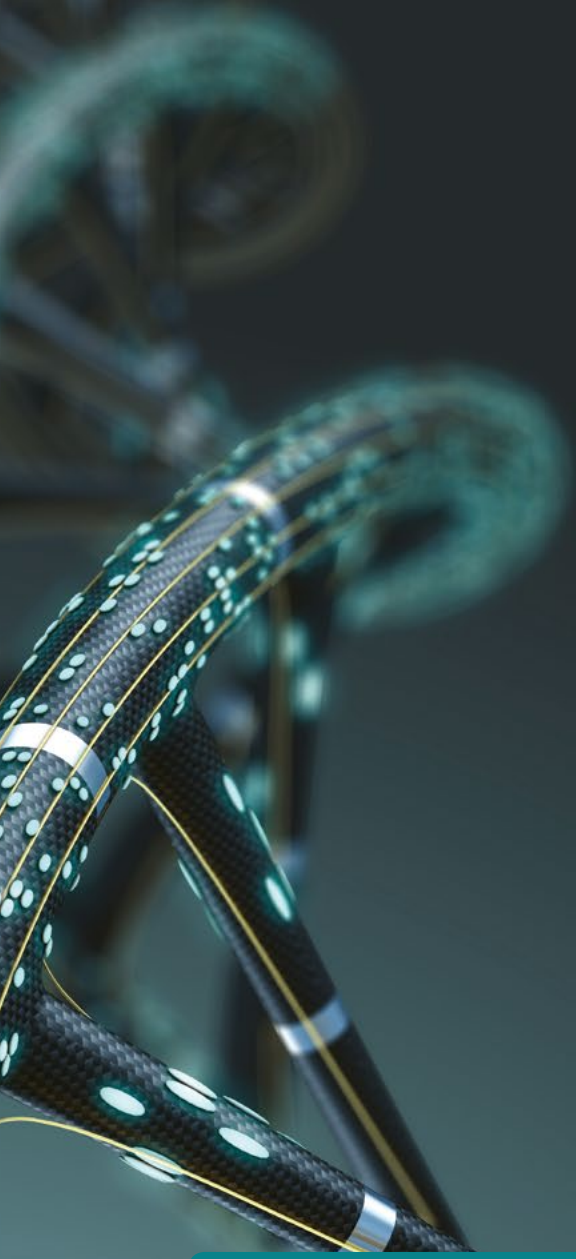
SUPPORTING SERVICES

In addition to the main investigative assays in SOLVO's portfolio (as listed above), a number of supporting test and services are also provided to ensure our service experiments are run at the most optimal conditions adapted to each test article (TA). These services are mostly offered as part of bigger study packages but can also be requested as standalone assays if needed.

Service	Main approach*
Plasma Protein Binding (PPB) Assessment	Unbound fraction determination using the Rapid Equilibrium Dialysis (RED) approach with different plasma or matrix proteins (e.g., serum albumins or full serum), or cellular elements.
Solubility tests	Compound solubility tests in the relevant assay buffers using microscopic visual inspection or nephelometry.
Non-specific binding (NSB) assessment	Additional steps to assess TA recovery or separate measurements to test NSB to cells or plasticware can be added with most assay types.
Cytotoxicity tests	Detection of cell death after incubation (via resazurin-based cytotoxicity test or LDH-release) or monolayer integrity assessment (TEER, permeability reference controls).
Bioanalytical (BA) services	LC/MS method development and validation and compound quantification in assay samples for several modalities. Complexity adapted to project: from screens to regulatory compliant BA reports. ICP-MS quantification available. Purity check for radiolabeled moieties.
Quality Check of assays*	All studies** are run with at least Discovery QC, Regulatory QC and Extended Regulatory QC can be requested and are recommended for studies intended for regulatory submission.

*To learn more about our QC activities, archiving and reporting options, please consult with your local SOLVO representative.
**Except for custom development projects.

For further information on assay setups, availability or with any specific requests, please contact us at solvobiotech@crl.com to consult with our experts!



PRICES ARE AVAILABLE UPON REQUEST AT **SOLVOBIOTECH@CRL.COM**

SOLVO's study design is flexible. The main experimental parameters, including probe substrate, reference inhibitor, and number of concentrations or replicates can be changed according to individual requirements.

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