SOLVO®
BIOTECHNOLOGY
A CHARLES RIVER COMPANY

IN VITRO ADME AND TRANSPORTER SERVICES

1.

MEMBRANE TRANSPORTER SERVICES

SOLVO's transporter assays are available in predefined assay setups that can be tailored for the specific needs of any project, including screening-type studies. For most transporter assays, multiple probe substrate and reference inhibitor 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 into or across certain tissues and contributing to their active removal from cells.

A compound's interaction with efflux transporters can be studied in 2 different assay systems.

Bidirectional Permeability Assays

Bidirectional Permeability or so-called Monolayer Assays are performed using polarized cell monolayers overexpressing the efflux transporter of interest via stable transfection (e.g., 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. Selection of the appropriate system, assay setup and controls for assessing a specific test article as transport substrate or inhibitor, is driven by compound- and project-specific parameters.

Human Efflux Transporters

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

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
	Rat	
	NHP (cynomolgus monkey)	
Bsep	Mouse	Vesicular Transport assay
	Rat	
	NHP (cynomolgus monkey)	
	Dog	
Mrp2	Rat	

*Transporters are expressed in different cell lines, assays were characterized and are offered often with multiple probe substrates and inhibitors. For details, 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.

A compound's 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 (cynomolgus monkey)	Dog
mouseSglt2	ratAsbt	cyNtcp	dogSglt2
	ratNtcp	cyOatp1b1	
	ratOat1	cyOatp1b3	
	ratOatp1a1	cyOatp2b1	
	ratOatp1a4		
	ratOatp1b2		
	ratOctn2		
	ratOstα/β		

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

Bidirectional permeability assays with multiple transporters

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 Permeability Assays		
Transporters	**Cell line**	**Barrier modeled**
MDR1	Caco-2 (or C2BBe1-BCRP-KO)	Intestinal, BBB, general permeability
BCRP	Caco-2 (or C2BBe1-BCRP-KO)	
MRP2	Caco-2 (or C2BBe1-MRP2-KO)	
Double Transfected Permeability 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 Permeability Assays		
Transporters	**Cell line**	**Barrier modeled**
-	MDCKII-Abcb1-KO	Passive permeability assessment
-	Parental MDCKII	Canine Mdr1 (Abcb1) assays

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 characterization. CYP450-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.

Custom assay development

For transporter interaction assessment and related ADME-Tox services beyond our standard portfolio, custom assay development is also offered as a service, carried out by our R&D team. For transporter interactions, fully characterized 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 cells can be generated for qualitative interaction assessment.

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 test compound at multiple concentrations in microsomes with and without NADPH. The assay is performed using a cocktail of 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 levels (measured by RT-qPCR), and/or in CYP activity (measured by LC-MS/MS) using a specific probe substrate.

Service*	System	Species	Enzymes	Endpoints
CYP induction	Cryopreserved plateable human hepatocytes	Human	CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4	Fold-change in mRNA levels, Fold-change in CYP activity, EC_{50} and E_{max}: TA recovery, Cytotoxicity assessment (LDH)
	Cryopreserved plateable rat hepatocytes	Rat	Cyp1a1, Cyp2b1, Cyp2c11, Cyp3a1	

*Number of donors and endpoints 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 preclinical species. The test compound is incubated with the enzyme source (hepatocytes, or subcellular fractions with co-factors) and its presence in the assay system is monitored over time. Appropriate controls are included to ensure distinction of metabolism-dependent loss of substrate from chemical degradation or non-specific binding. Compound half-life (min) and intrinsic clearance (µL/min per mg protein or per million cells) are 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 characterization

Metabolite characterization 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 metabolite (if any) 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 characterization	Cryopreserved suspension hepatocytes, pooled liver microsomes or S9 fraction	Human, cynomolgous monkey, dog, rat, mouse	various	Structures proposed for putative metabolites (if any)

3.

BARRIER MODEL SERVICES

The use of more complex *in vitro* barrier model systems allows the assessment of compound interactions in a more physiological-like context. Cells directly isolated from different barrier organs such as hepatocytes, renal proximal tubule cells or brain endothelial cells can be maintained in different assay formats that allow at least partial conservation of *in vivo* tissue functions, including transporter activity. These systems thus allow the *in vitro* study of multiple mechanisms simultaneously in an organ-specific environment.

Hepatocyte uptake assays

Hepatocyte uptake assays utilize cryopreserved primary hepatocytes to investigate temperature-, time- and concentration-dependent intracellular accumulation of compound (e.g., small molecules, oligos, peptides, etc.). Interactions with transporters expressed in hepatocytes (such as OAT2, OATP1B1, OATP1B3, OCT1, etc.; or their preclinical species orthologs) can also be tested. SOLVO characterized the assays for human, cynomolgus monkey, dog, rat and mouse hepatocytes. The assay also allows for K_M/V_{max} 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 and Matrigel, 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 in human or rat hepatocytes. Additionally, with a modified experimental setup, basolateral, biliary efflux and intracellular accumulation of the parent compound and metabolites can also be determined at the same time.

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 and metabolic enzyme expression and activity over several weeks in culture. Potential applications, using hepatocytes from human or preclinical species, 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 available for studying renal interactions in human and certain preclinical species.

Brain Endothelial Cell (BEC) Monolayer Assays

The Brain Endothelial Cell monolayer assays using procine-, rat- or mouse cells 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, as well as transporters. The BEC monolayer assay is a great tool for predicting or corroborating *in vivo* brain permeability ($K_{p,uu}$) data, assessing cytotoxic effects, and other potential project-specific applications may also be supported.

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, NHP (Cyno. monkey), Dog, Rat, Mouse
Hepatocyte Uptake Assays – Oil-spin method	Human, NHP (Cyno. monkey), Dog, Rat, Mouse
Sandwich-Cultured Hepatocyte (SCH) Assays	Human, Rat
HepatoPac Metabolite Characterization – long term incubation	Human, Dog, Mouse
HepatoPac Metabolic Stability – long term incubation	Human, Rat
HepatoPac Toxicity – long term incubation	
Renal Proximal Tubule Cell (PTC) Monolayer Services	Human, NHP (Cyno. monkey), Rat
Rodent Brain Endothelial Cell (BEC) Monolayer Assays	Porcine, Rat, Mouse
BCS-based Permeability Classification (Caco-2 model)	Human

*Additional species may be available upon request.

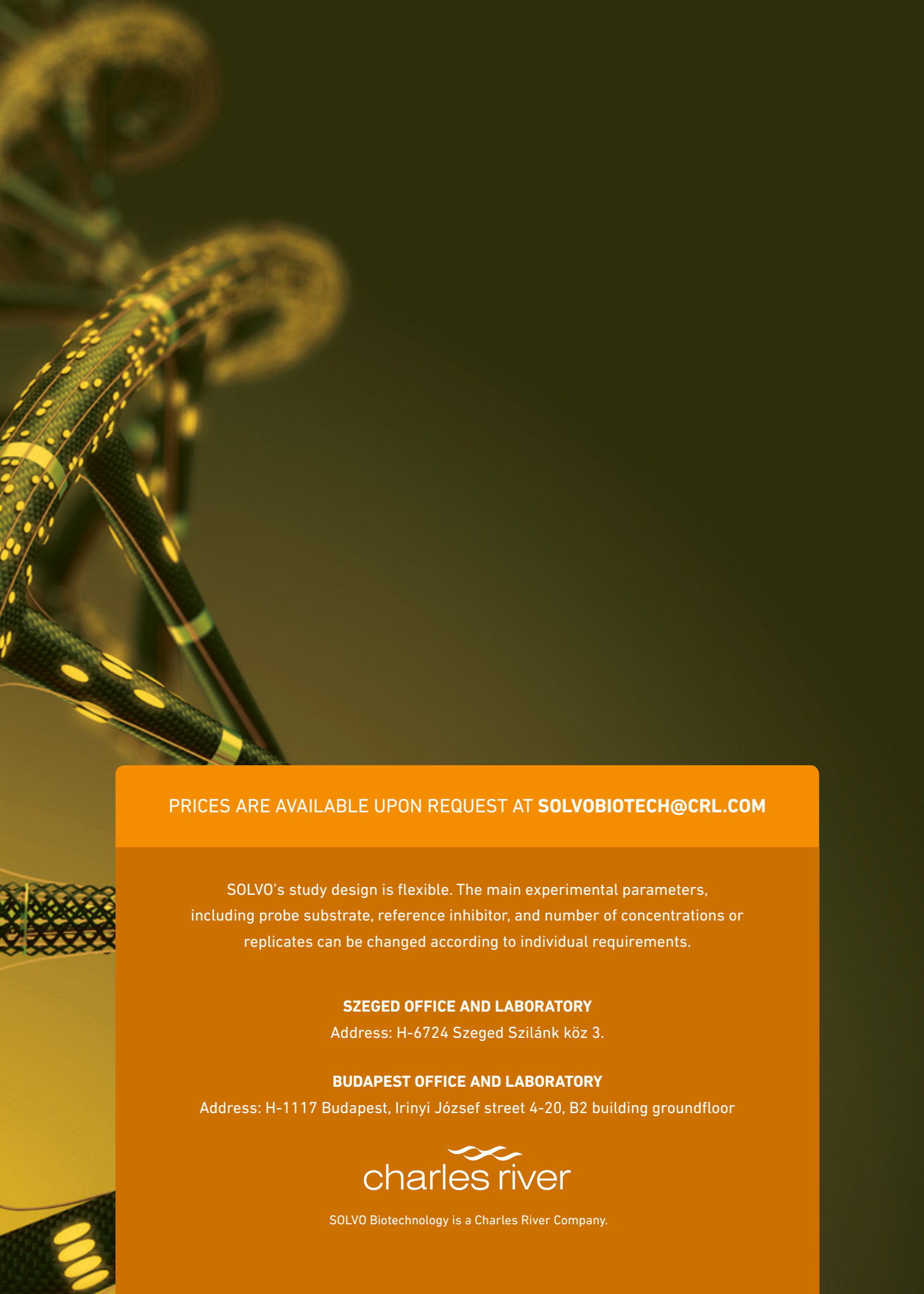
4.

SUPPORTING SERVICES

A number of supporting test and services are provided to ensure our service assays are conducted at the most optimal conditions specifically determined for each tests article (TA). These services are mostly offered as part of larger 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.
Cell viability tests	Detection of cell death after incubation (via resazurin-based cytotoxicity test or LDH-release) or monolayer integrity assessment (permeability reference controls, TEER measurement).
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.

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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