

ADME-TOX PROFILES

■ DRUG SCREENING

■ OPTION I (BIOAVAILABILITY)

This ADME-Tox profile provides useful compound bioavailability information. This profile combined with the ADME-Tox profile-option II and with pharmacology profiling data will help optimize drug candidate selection.

Ref. P12	ASSAY	DETECTION	TEST CONCENTRATION	REF.
🕒 2 weeks	Aqueous solubility	HPLC-UV/VIS	200 µM (n=2)	0435
	Partition coefficient (Log D, n-octanol/PBS, pH 7.4)	HPLC-UV/VIS	100 µM (n=3)	0417
	Plasma protein binding (equilibrium dialysis)	🚫 HPLC-MS/MS	10 µM (n=2)	2194
	A-B permeability (TC7, pH 6.5/7.4)	🚫 HPLC-MS/MS	10 µM (n=2)	0423
	Metabolic stability (human liver microsomes)	🚫 HPLC-MS/MS	1 µM (n=2)	0416
	HPLC-MS screen	HPLC-MS/MS	200 µM (n=1)	0881

■ OPTION II (LEAD SELECTION/PRIORITIZATION)

This ADME-Tox profile focuses on early safety evaluation by providing drug-drug interaction, cytotoxicity and cardiotoxicity information. This profile combined with the ADME-Tox profile-option I and with pharmacology profiling data is a rapid cost-effective way for prioritizing drug candidates.

Ref. P13	ASSAY	DETECTION	TEST CONCENTRATION	REF.
🕒 2 weeks	P-gp inhibition (calcein AM substrate)	🚫 fluorimetry	1, 30, 100 µM (n=2)	1324
	CYP1A2 inhibition (recombinant, CEC substrate)	🚫 fluorimetry	10 µM (n=2)	0389
	CYP2C9 inhibition (recombinant, MFC substrate)	🚫 fluorimetry	10 µM (n=2)	0412
	CYP2C19 inhibition (recombinant, CEC substrate)	🚫 fluorimetry	10 µM (n=2)	0390
	CYP2D6 inhibition (recombinant, MFC substrate)	🚫 fluorimetry	10 µM (n=2)	1338
	CYP3A4 inhibition (recombinant, BFC substrate)	🚫 fluorimetry	10 µM (n=2)	0391
	hERG (functional)	🚫 automated patch-clamp	0.1, 1, 10 µM (n=2)	2245
	cytotoxicity panel (HepG2)	🚫 high-content analysis	1, 30, 100 µM (n=2)	1537

■ GENOTOXICITY PROFILE

For early genetic toxicity assessment, this package includes the Ames fluctuation assay, evaluating the mutagenic potential of chemicals and the *in vitro* micronucleus assay in CHO-K1 cells complementing the Ames test in the evaluation of genotoxic effects like chromosomal damage.

Ref. P14	ASSAY	DETECTION	TEST CONCENTRATION	REF.
🕒 2 weeks	Ames, TA98, TA100 and TA1535 (± S9) 🚫	photometry	5, 10, 50, 100 µM (n=48)	1899
	<i>in vitro</i> micronucleus, CHO-K1 (± S9)	high-content analysis	1 to 500 µM (n=2)	1900

DRUG DEVELOPMENT

The following CYP-based drug-drug interaction profile and P-gp mediated drug-drug interaction profile have been designed following the latest FDA draft guidance*.

These profiles comprise several modules, which can be purchased as a full panel or by module.

* FDA/CBER guidance for Industry on Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling; available on the internet at <http://www.fda.gov/cber/gdlns/interactstud.htm>

CYP-BASED DRUG-DRUG INTERACTION PROFILE

These assays will answer the questions in lines 177-197 of the guidance: whether a test compound is metabolized by and/or inhibits/induces any of these CYPs. Negative findings from this profile could eliminate the need for later clinical investigations (however, the negative finding should be interpreted in context, e.g. if *in vivo* concentration of a test compound is greater than 10 µM, our negative finding in CYP inhibition at 10 µM may not eliminate the need for further *in vivo* study).

THE CYP-BASED DRUG-DRUG INTERACTION PROFILE CONTAINS 3 MODULES:

1 CYP PHENOTYPING

Human recombinant CYPs are used to evaluate whether the test compound is specifically metabolized by one or more of the isozymes tested.

(10 assays)

2 CYP INHIBITION

Using unique, CYP specific substrates, the test compound is evaluated for its ability to inhibit one or more of the isozymes tested within the context of a human liver microsome matrix.

(10 assays)

3 CYP INDUCTION

Test concentrations used are defined around C_{max}. The CYP1A, CYP2B6, and CYP3A enzyme activities are measured in hepatocytes derived from three different human donors to address the donor variability.

(3 assays)



FULL PANEL/3 modules

Ref. **P19**

🕒 3 weeks

Module **1** Ref. **P19-m1**

🕒 2 weeks

Module **2** Ref. **P19-m2**

🕒 2 weeks

Module **3** Ref. **P19-m3**

🕒 3 weeks

FDA DRAFT GUIDANCE

ASSAY	DETECTION	TEST CONCENTRATION	REF.
MODULE 1 CYP PHENOTYPING			
metabolic stability (CYP1A2)	🚫 HPLC-MS/MS	1 µM (n=2)	1604
metabolic stability (CYP2A6) 🕒🚫	🚫 HPLC-MS/MS	1 µM (n=2)	3120
metabolic stability (CYP2B6)	🚫 HPLC-MS/MS	1 µM (n=2)	2226
metabolic stability (CYP2C8)	🚫 HPLC-MS/MS	1 µM (n=2)	2043
metabolic stability (CYP2C9)	🚫 HPLC-MS/MS	1 µM (n=2)	1602
metabolic stability (CYP2C19)	🚫 HPLC-MS/MS	1 µM (n=2)	1603
metabolic stability (CYP2D6)	🚫 HPLC-MS/MS	1 µM (n=2)	0716
metabolic stability (CYP2E1)	🚫 HPLC-MS/MS	1 µM (n=2)	2706
metabolic stability (CYP3A4)	🚫 HPLC-MS/MS	1 µM (n=2)	0715
metabolic stability (CYP3A5)	🚫 HPLC-MS/MS	1 µM (n=2)	1362
MODULE 2 CYP INHIBITION			
CYP1A (HLM, phenacetin substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	2064
CYP2A6 (HLM, coumarin substrate) 🕒🚫	🚫 HPLC-MS/MS	10 µM (n=2)	3119
CYP2B6 (HLM, bupropion substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	2065
CYP2C8 (HLM, paclitaxel substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	2244
CYP2C9 (HLM, diclofenac substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	2066
CYP2C19 (HLM, omeprazole substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	1772
CYP2D6 (HLM, dextromethorphan substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	1838
CYP2E1 (HLM, chlorzoxazone substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	2068
CYP3A (HLM, midazolam substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	1770
CYP3A (HLM, testosterone substrate)	🚫 HPLC-MS/MS	10 µM (n=2)	1769
MODULE 3 CYP INDUCTION			
CYP1A (human hepatocytes)	🚫 HPLC-MS/MS	3 concentrations (n=3) (3 individual donors)	2401
CYP2B6 (human hepatocytes)	🚫 HPLC-MS/MS	3 concentrations (n=3) (3 individual donors)	2403
CYP3A (human hepatocytes)	🚫 HPLC-MS/MS	3 concentrations (n=3) (3 individual donors)	2402

■ P-gp MEDIATED DRUG-DRUG INTERACTION PROFILE

P-glycoprotein (P-gp) is an efflux pump located in the intestine and blood-brain barrier among other tissues. Compounds that are substrates for P-gp may be secreted back into the lumen of the intestine. Additionally, compounds may be inhibitors of P-gp, and interfere with the efflux of concurrently administered drugs, resulting in potentially toxic levels and severe side effects. Compounds that are P-gp substrates may or may not inhibit P-gp activity. Similarly, compounds that are P-gp inhibitors may or may not be substrates for P-gp. The FDA draft guidance for industry on drug interaction studies addresses study design and data analysis for evaluating P-gp substrates and inhibitors. It recommends the bi-directional transport assay as the definitive assay for identifying P-gp substrates and inhibitors since this measures drug efflux in a more direct manner than other methods.

The P-gp-mediated drug-drug interaction profile is a set of assays designed to comply with the FDA draft guidance recommendations.

THE P-gp MEDIATED DRUG-DRUG INTERACTION PROFILE CONTAINS 2 MODULES:

1 P-gp SUBSTRATE EVALUATION

Step I assays are designed to assess a compound's non-specific binding and determine the efflux ratio and linear flux range.

Step II assays are designed to confirm whether the efflux activity is related to P-gp by including P-gp inhibitors and to evaluate the concentration dependence of the test compound.

(7 assays)

2 P-gp INHIBITOR EVALUATION

Step I assays are designed to assess a compound's range of P-gp inhibitory concentrations.

Step II assays are designed to determine the IC₅₀ value of the test compound.

(3 assays)



FULL PANEL/2 modules

Ref. **P21**

24-well format

4 weeks

Module **1** Ref. **P21-m1**

Module **2** Ref. **P21-m2**

4 weeks

FDA DRAFT GUIDANCE

ASSAY	DETECTION	TEST CONCENTRATION
MODULE 1 P-GP SUBSTRATE EVALUATION		
Step I/Preliminary verification (5-time points)		
A-B permeability (blank filter, 2 time points)	HPLC-MS/MS	1 µM (n=3)
A-B permeability (TC7)	HPLC-MS/MS	1 µM (n=3)
B-A permeability (TC7)	HPLC-MS/MS	1 µM (n=3)
Step II/Full evaluation (2 time points)		
A-B permeability (TC7) + inhibitor ¹	HPLC-MS/MS	1 µM (n=3)
B-A permeability (TC7) + inhibitor ¹	HPLC-MS/MS	1 µM (n=3)
A-B permeability (TC7)	HPLC-MS/MS	1, 10, 100 µM (n=3)
B-A permeability (TC7)	HPLC-MS/MS	1, 10, 100 µM (n=3)
MODULE 2 P-gp INHIBITOR EVALUATION		
Step I /Preliminary verification		
P-gp inhibition B-A	liquid scintillation counting	10, 100, 500 µM (n=3)
Step II / Full evaluation		
P-gp inhibition B-A	liquid scintillation counting	7 concentrations ² (n=3)
P-gp inhibition A-B	liquid scintillation counting	7 concentrations ² (n=3)

¹ Inhibitors include ketoconazole at 50 µM and verapamil at 100 µM.

² Concentrations based on results in Step I.

SAMPLE SIZE

Values are based on a molecular weight ≤ 500 g/mol and assays tested at default concentrations.

	Number of assays ▼	SCREENING	
		WEIGHT (pre-weighed)	VOLUME (100% DMSO)
ADME-TOX PROFILES			
Option I (Bioavailability)	6	1 - 2 mg	100 μ L@10 mM
Option II (Lead selection/Prioritization)	8	1 - 2 mg	100 μ L@10 mM
Genotoxicity profile	2	5 mg	200 μ L@50 mM
CYP-based drug-drug interaction profile	23 ²	10 mg	–
P-gp mediated drug-drug interaction profile	10 ²	20 mg	–

¹ Follow-up with highest concentration at 10 μ M (see page 12 for highest concentration at 100 μ M) – Assuming ~10% of test in IC₅₀. Usually, for 1 IC₅₀: 30 μ L@10 mM and + 25 μ L@10 mM by additional IC₅₀.

² Full panel. For compound amount needed for separate modules: please inquire



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ASSAY CATALOG REFERENCES

In 2009, Cerep finalized one of the milestones in the industrialization process: the implementation of new referencing and supply chain management systems. The references of each of the assays have thus been simplified: **each reference will now be displayed as 4 digits.**

A correlation table between old and new assay references is available at [www.cerep.com CATALOG ONLINE](http://www.cerep.com/CATALOG_ONLINE)
<<http://www.cerep.com/Cerep/Users/pages/Catalog/Assay/catalog.asp>>

QUESTIONS OR CONCERNS?

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