

CYP Inhibition Assay

As Cytochrome P450 (CYP) enzymes are the primary drug metabolizing enzymes in the body, they play a significant role in the metabolic clearance of drugs. CYP inhibition occurs when a new drug inhibits the metabolism of other drugs, which may lead to:

- Increased drug concentrations in plasma and tissue
- Reduction of drug clearance
- Increased drug toxicity

Your Partner in CYP Inhibition Assays

Our services team offers a standardized protocol for assessing direct, indirect, and time-dependent CYP inhibition according to the *FDA Guidance document entitled, In Vitro Metabolism and Transporter Mediated Drug-Drug Interaction Studies**. This ensures high-quality data with the speed and accuracy needed for early drug discovery. We provide study designs suitable for IND submissions and collaborate with you to tailor the study to your specific research needs.

Benefits of Our Services:

Accurate and reliable data for informed decision-making

Fast turnaround times to keep your drug development on track

FDA guidance-based studies to meet regulatory requirements

Collaborative approach to ensure the study addresses your research questions

Additional LifeNet Health LifeSciences Services | Click to learn more:

- In vitro assay services
- DMPK/ADMET assays
- OECD safety studies
- Cytotoxicity assays

Standard Protocol for In Vitro CYP Inhibition (IC50 and Metabolite-dependent IC50 shift)

ASS

Standard Protocol for Determination of Time-Dependent Inhibition (K_{inact} and KI)

ASSAY PARAMETER	PROTOCOL	ASSAY PARAMETER	PROTOCOL
Test system (panel of donors for client selection)	Human liver microsomes (pooled) (rat, dog, and NHP also available)	Test system (panel of donors for client selection)	Human liver microsomes (pooled) (rat, dog, and NHP also available)
Plate format	96-well	Microsomal protein concentration	0.1mg/mL
Number or reaction setups	3 (no inhibitor drug, 30 min pre incubation without NADPH, 30 min pre incubation with NADPH)	Plate format	96-well
Replicates	3	Enzymes evaluated	CYP1A2 (phenacetin), CYP2B6 (bupropion), CYP2C8 (paclitaxel), CYP2C9 (diclofenac), CYP2C19 (S-mephenytoin), CYP2D6 (dextromethorphan), CYP3A4 (midazolam)
Reference compounds/ controls direct inhibitors	1 (α-naphthoflavone, sertraline, itraconazole)	(probe drugs)	
Reference compound metabolism dependent inhibitors	1 (furafylline, clopidogrel, azamulin)	Replicates	2
Micosomal protein concentraion	0.1mg/mL	Reference compounds/control	Diltiazem (additional controls can be added)
Test compound concentrations (can be adjusted)	6 (.01, 0.1, 1.0, 2.0, 5.0, 10.0, μΜ)	Test compound concentrations (can be adjusted)	6 (.01, 0.1, 1.0, 2.0, 5.0, 10.0, μΜ) plus 0
Exposure time	10 min	Exposure times	6 plus 0 (typically 5-30 min)
Amount of test compound required	10 mg	Amount of test compound required	10 mg
Analytical method (substrate drug)	LC/MS/MS	Analytical method (substrate/probe drug)	LC/MS/MS
CYP-Glo Promega	Luminescence	Time to complete	2-3 weeks
Time to complete	2-3 weeks	Regulatory	Non-GLP or GLP
Regulatory	Non-GLP or GLP	Deliverables	Kl (µM), K _{inact} (min⁻¹), K _{inact} /Kl (mL/min/µmole), full report, graphs/tables
Deliverables	IC50, shifted IC50, full report, graphs/tables		
Note: IC50 values are dependent on the substrate concentration for competitive inhibitors, and not for non-competitive inhibitors			

List of FDA recommended CYP Targets and their Substrates

CYP ENZYME TARGET (ISOFORM)	SUBSTRATE DRUG MARKER REACTION	
СҮР2В6	Bupropion hydroxylation	
СҮРІА2	7-ethoxyresorufin-O-deethylation	
CYP2C8	Amodiaquine N-deethylation	
СҮР2С9	Diclofenac 4-hydroxylation	
СҮР2С19	S-Mephenytoin 4-hydroxylation	
CYP2D6	Bufuralol 1-hydroxylation or Dextromethorphan O-demethylation	
CYP3A4/5	Midazolam 1-hydroxyltaion and testosterone 6β-hydroxylation	

Note: The metabolite of each substrate (marker) drug will be measured by LC/MS/MS. It is also possible to use luciferin-substrates (marker) for each CYP enzyme and monitor metabolism by luminescence.

How can we help?

Talk with one of our experts for help with general inquiries, protocol details, or becoming a new client.

LNHlifesciences.org/information 1-888-847-7831 (US & Canada) cells_tissues@lifenethealth.org