Cytochrome P450 Inhibition Assay (fluorogenic, panel of 5 CYP450)

Background: Cytochrome P450 (CYP) enzymes represent a heme-containing protein superfamily metabolizing a broad variety of xenobiotics, including drugs and toxic chemicals. 11 CYP families are expressed in a human liver and gastrointestinal tract (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP3A4/5), and 5 of them (CYPs 1A2, 2C9, 2C19, 2D6 and 3A4) are involved in about 95% of the known drug metabolism. Cytochrome P450s are of critical importance due to the two of the most significant problems in clinical pharmacology: metabolismmediated drug-drug interactions (DDI) and individual variability in drug metabolism. Most drugs undergo deactivation by CYPs, either directly or by facilitated excretion from the body. Some substances are bioactivated by CYPs to form pharmacologically active compounds. Also, many drugs may increase or decrease the activity of various CYPs due to the ability of binding to them. It is important to evaluate the potential inhibition of a new drug candidate for the most clinically relevant CYP450 enzymes. CYP450 inhibition may potentially lead to elevated in vivo plasma levels of a co-administered drug metabolized by the inhibited enzyme, and, consequently, to adverse drug reactions and toxicity. During the early stages of drug discovery process, routine assessment to identify the following major CYP enzymes for potential metabolism-mediated interactions is recommended: CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.

Service Details: The high-throughput fluorogenic CYP450 inhibition assay is fast and cost-effective method most frequently applied during drug discovery process. The potential for CYP450 inhibition is assessed by performing in vitro inhibition studies using specific fluorogenic CYP450 substrates with the corresponding individually expressed CYP450 enzymes and NADPH regeneration system (Vivid® CYP450 Screening Kits). The fluorogenic probes are transformed by CYP450 to give fluorescent compounds (hydroxycoumarin or resorufin analogues). The fluorescent signal produced from reaction is directly proportional to the cytochrome P450 activity. In the cases when tested compounds interfere with the CYP450 enzyme-substrate reaction, the fluorescent signal decreases, which is detected using fluorometric multi-well plate reader.

CYP450	Substrate	Reference Inhibitor
1A2	EOMCC	α-Naphthoflavone
3A4	BOMCC	Ketoconazole
2C9	OOMR	Sulphaphenazole
2C19	EOMCC	Ticlopidine
2D6	MOBFC	Quinidine

For the rough estimate, single point assays are typically performed for each compound at 10 μM concentration or another concentration stipulated by the customer. Reference inhibitors specific for each CYP450 enzyme are used to assess CYP450 inhibition in the control experiments for every batch of tested compounds. Test concentrations of the reference compounds correspond to approximately 50x fold reported IC50 values for the corresponding cytochromes P450, which is expected to produce 80-100% inhibition in the properly performing assay. The negative control (baseline) does not contain CYP450 enzyme. If a noticeable inhibition is detected, the IC50 values for the tested compounds can be determined upon request. For this purpose, 8-point, 3-fold serial dilution dose-response inhibition curves of the compounds are built, starting at 100 μM (or another) compound concentration. Based on this data, IC50 values for a compound for each of the CYP450 enzymes are calculated using GraphPad Prism software. All test points are performed in quadruplicates. Considering that interference can occur from the test compound exhibiting intrinsic fluorescence or fluorescence quenching, which can lead to the false results, it is recommended to test them using alternative LC-MS/MS based assay.

Deliverable: Either single point assay data for each compound at 10 μM concentration or IC50 values based on 8-point, 3-fold serial dilution dose-response inhibition curves (upon request). Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 μmol) or 100 μL of 10 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient, which should be discussed for each particular project.