

# Promega's P450-Glo™ Luminescent Cytochrome P450 Assay Using the FLUOstar OPTIMA

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- Screen for cytochrome P450 inhibitors using Promega's P450-Glo™ assay on BMG LABTECH's FLUOstar OPTIMA
- The FLUOstar OPTIMA's dedicated luminescence detection system gives a strong signal and minimizes background noise
- Rapid and reliable detection of cytochrome P450 activity

## Introduction

A large group of enzymes known as cytochromes P450 (CYP or P450) are responsible for the oxidative metabolism of many xenobiotic, hydrophobic chemicals, which include most therapeutic drugs<sup>1</sup>. P450 metabolism can influence the clearance rate of drugs, their toxicity, and their interactions with other drugs. For drug discovery, researchers need to determine how new drug molecules are metabolized by P450 enzymes and how they may alter P450 activity. Some of these determinations can be achieved by using known P450 substrates as probes that change in a measurable way when they react with a P450<sup>2</sup>. If the reactivity of the probe is altered in the presence of a drug, it can be concluded that the drug has an impact on P450 activity. As described herein, novel P450 inhibitors can be found if they reduce the reactivity of a P450 enzyme with a probe substrate.

BMG LABTECH's FLUOstar OPTIMA (Figure 1) is a flexible multifunctional microplate reader that has four different measurement modes in one instrument: fluorescence intensity, time-resolved fluorescence, high-performance luminescence and absorbance. All BMG LABTECH OPTIMA series of reader, as well as the PHERAstar, are equipped with a dedicated luminescence detection system. This system allows for the measurement of a strong luminescent signal, while keeping the background noise low. The FLUOstar OPTIMA can also be easily equipped with injectors that deliver reagent at the point of measurement, thereby allowing the unit to perform flash luminescence assays.



Fig. 1: FLUOstar OPTIMA multifunctional microplate reader

## Assay Design

P450-Glo™ Assays employ luminogenic P450 probe substrates that are derivatives of beetle luciferin, a substrate for luciferase

enzymes. The derivatives are not substrates for luciferase but are converted by different P450 isoenzymes to luciferin (Figure 2), which in turn reacts with luciferase to produce a measurable amount of light that is directly proportional to the P450 activity. A luminogenic substrate is incubated with an active P450 preparation. The P450 activity is stopped and luciferin detected by adding Ultra-Glo™ Luciferase, a recombinant, stable luciferase in a proprietary buffer system that generates a "glow-type" luminescent signal (Figure 3). The half-life of the luminescent output is greater than two hours, allowing batch plate processing.

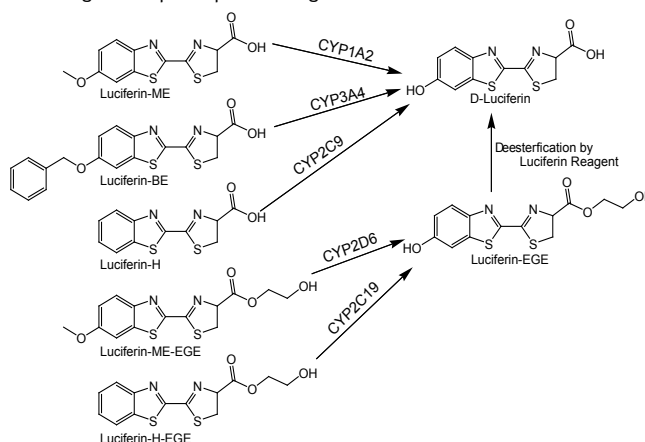


Fig. 2: P450-Glo™ luminogenic substrates converted by specific CYP isoenzymes to luciferin. (ME = methyl ether; BE = benzyl ether; EGE = ethylene glycol ether)

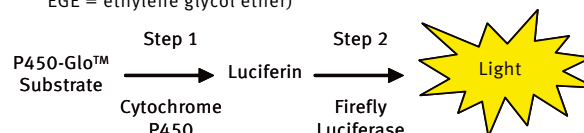


Fig. 3: P450-Glo™ luminescent assay steps

## Materials and Methods

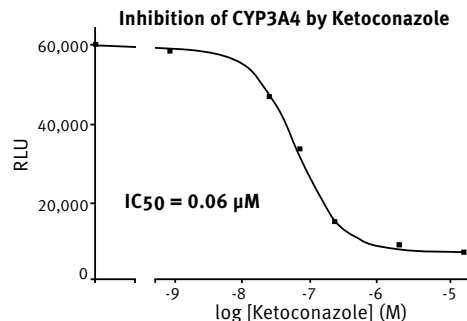
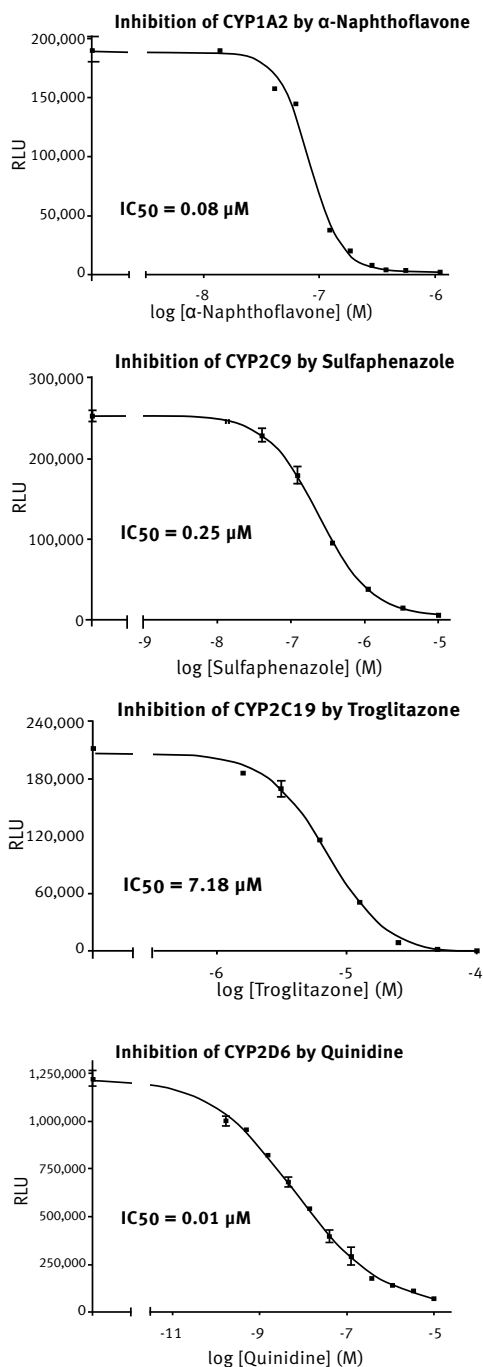
- BMG LABTECH FLUOstar OPTIMA
- Opaque, flat-bottom 96-well Costar® plates
- Promega's P450 Glo™ Screening System (contains baculovirus membrane preparation with human cytochrome P450 enzyme, luminogenic substrate (Fig. 2), NADPH regeneration solution, luciferin detection reagent, reaction buffer, and luciferin-free water) for: CYP1A2 (catalog # V9770), CYP2C9 (#V9790), CYP3A4 (#V9800), CYP2C19 (#V9880), and CYP2D6 (#V9890)

P450 assays were done with membrane preparations from insect cells co-expressed with P450 reductase or P450 reductase plus cytochrome b5. For background measurements membrane fractions with no P450 enzymes were used. P450 reactions were performed in opaque 96-well Costar® plates using a 50µl volume in KPO<sub>4</sub> buffer (pH 7.4). P450 enzymes were incubated with their substrates for 10-30 minutes at 37°C. Reactions were initiated by the addition of an NADPH regenerating solution. P450 activity was stopped and luminescence was initiated by adding Luciferin Detection Reagent<sup>3</sup>. Luminescence was read directly on the

FLUOstar OPTIMA microplate reader in luminescence mode. The instrument's incubation chamber and onboard injectors can be easily used to automate the process. Each data point represents the average of three wells.

## Results and Discussion

Using the BMG FLUOstar OPTIMA, P450-Glo™ Assays can detect dose-dependent inhibition of recombinant P450s by known P450 inhibitors. Figure 4 shows dose related inhibition activity curves of the top five drug metabolizers (CYP1A2, 2C9, 2C19, 2D6, and 3A4) and their respective inhibitors. The determined IC<sub>50</sub> values are similar to values obtained with conventional probe substrates<sup>4</sup>. This data demonstrates that a range of concentrations of a single compound can be screened to measure IC<sub>50</sub> values, but it also implies that single concentrations of multiple test compounds can be screened against a chemical library.



**Fig. 4:** P450-Glo™ IC<sub>50</sub> calculations for CYP450 enzymes (CYP1A2, 2C9, 2C19, 2D6, and 3A4) and their respective inhibitors ( $\alpha$ -naphthoflavone, sulfaphenazole, troglitazone, quinidine, and ketoconazole).

## Conclusion

P450-Glo™ Assays provide a rapid, sensitive and highly reproducible approach to P450 screening. The systems are compatible with conventional P450 assay protocols in automated or manual multiwell plate formats. P450-Glo™ Luminescent Assays have several unique properties:

- Speed - The luminescent reaction is immediate and eliminates the need for time-consuming analyses
- Great Sensitivity - Low background levels and a large dynamic range result in sensitive assays that require less P450 enzyme
- Soluble Substrates - P450-Glo™ substrates are highly soluble in aqueous solutions.
- Single readout - Multiple P450 isoforms with multiple substrates can be assayed at a single instrument setting.
- Low false-positive rate - Proprietary stabilized firefly luciferase minimizes the number of false positives due to inhibition of luciferase by analytes when screening for P450 inhibitors.

BMG LABTECH's FLUOstar OPTIMA microplate reader provides the perfect platform to perform Promega's P450-Glo™ Assay. With its dedicated luminescence detection system the FLUOstar minimizes the background signal, thereby increasing the confidence in the data. Furthermore, with two optional onboard injectors and a standard 45°C incubation chamber, the FLUOstar OPTIMA can easily become fully automated to perform all of your P450 screening needs. The experiments described herein were performed with the FLUOstar OPTIMA; however, BMG LABTECH's LUMIstar OPTIMA, POLARstar OPTIMA, and PHERAstar could all be used to analyze Promega's P450-Glo™ Assays. In fact the PHERAstar, along with a Deerac Fluidics liquid handling instrument, was successfully used to miniaturize the P450-Glo™ Assay into an HTS format<sup>5</sup>.

## References

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