P450-Glo™ Luminescent CYP450 Assay

Figure 1 – The P450-Glo™ assay was performed by incubating a luminogenic cytochrome P450 substrate with a cytochrome P450 enzyme and NADPH Regeneration System. The P450-Glo™ substrate is a new small molecule substrate designed to mimic the metabolism of real drug substrates by human liver microsomes or primary hepatocytes, allowing for high-throughput lead profiling in chemically diverse libraries. A 384-well assay format was used to demonstrate the ease of the combined procedure.

BioTek Instrumentation

Figure 2 – Precision™ XS Microplate Sample Processor

Figure 3 – Synergy™ Mx Monochromator-Based Multi-Mode Microplate Reader

Precision™ XS Compound Titration Method P450-Glo™ Assay Validation

Table 1 – Component concentrations and incubation times for P450-Glo™ assays.

Table 2 – IC50 values for compounds tested with P450-Glo™ assays

CYP450 Inhibitor Profiling

Introduction

The ability to determine the effects that lead compounds have on cytochrome P450 enzymes is an important part of today’s drug discovery process. One desired component of this process is the ability to profile compounds against multiple CYP450 enzymes using the same basic protocol. This is an easy, as easy to deploy compounds that will create accurate titration curves. We have demonstrated the automation of dose-response curves using BioTek’s Precision™ XS Automated Pipetting System. This system represents a fully automated platform for generating dose-response curves for lead compounds against multiple CYP450 enzymes using the same basic protocol. The Precision™ XS combines a single-channel sample processing head, an 8-channel pipetting head, and a multi-channel reagent dispenser in one instrument. The instrument was used to serially titrate compounds against each CYP450 enzyme isoform in 96-well microplates, and the IC50 values for each compound were determined using recombinant CYP isoforms 3A4, 2C9, and 2D6. It is important to assess metabolism for appropriate dosing, but also for establishing metabolism-related drug-drug interactions where one drug may affect the metabolism of another, leading to possible toxic effects. While the gold standard method for determining the effects of a compound on the metabolism of a drug substrate is an in-vitro assay using human liver microsomes or primary hepatocytes, this process is labor-intensive, time-consuming, and costly. Automation of luminescence-based CYP inhibition assays, using BioTek instrumentation, provides such a solution. The P450-Glo™ assays provide a convenient, high-throughput solution for lead profiling in chemically diverse libraries. A 384-well assay format was used to demonstrate the ease of the combined procedure.

Abstract

Most small molecule drugs are metabolized predominantly in the liver by cytochrome P450 (CYP) enzymes, particularly CYP isoforms 3A4, 2C9, and 2D6. It is important to assess metabolisms for appropriate dosing, but also for establishing metabolism-related drug-drug interactions where one drug may affect the metabolism of another, leading to possible toxic effects. While the gold standard method for determining the effects of a compound on the metabolism of a drug substrate is an in-vitro assay using human liver microsomes or primary hepatocytes, this process is labor-intensive, time-consuming, and costly. Automation of luminescence-based CYP inhibition assays, using BioTek instrumentation, provides such a solution. The P450-Glo™ assays provide a convenient, high-throughput solution for lead profiling in chemically diverse libraries. A 384-well assay format was used to demonstrate the ease of the combined procedure.

Figure 4 – Graphs showing linearity of Precision™ XS compound titration method

Figure 5 – Graphs showing linearity of Precision™ XS compound titration method

Conclusions

1. The Precision™ XS provides an easy-to-use solution to deliver accurate component concentrations for IC50 determination.
2. The accuracy of the optical system in the Synergy™ Mx is able to deliver dependable results regardless of the sample well and plate type.
3. Promega’s P450-Glo™ Screening Systems provide rapid, high-throughput inhibition assays when compared to conventional methods. Component conditions and incubation times can be varied in order to optimize each particular assay.
4. The combination of BioTek’s instrumentation, and Promega’s P450-Glo™ Screening Systems create an ideal solution for high-throughput, automated cytochrome P450 profiling.

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References


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