Abstract

Early assessment of cytochrome P450 (CYP) inhibition has become an essential component of drug discovery screening. The CYP3A4 enzyme is the most abundant enzyme in the liver and plays a major role in metabolizing many xenobiotics in humans. The Promega P450-Glo™ CYP3A4 Assay (Luciferin-IPA) offers an extremely sensitive, high throughput and specific luminescence assay for the examination of CYP3A4 inhibition with pooled Human Liver Microsomes. The Echo 555 liquid handler provides precise and accurate acoustic transfer free of cross-contamination risk. Here, we demonstrate optimization of the P450-Glo™ Assay with Human Liver Microsomes (HLM) in low volume format using the Echo 555 liquid handler. We show that the Echo 555 liquid handler can transfer microsomes, inhibitors and substrate in nanoliter volumes, providing a sensitive and robust small scale assay. IC₅₀ results for four known CYP3A4 inhibitors using our miniaturized assay compared favorably to previously reported values in the literature.

Introduction

The Labyco Echo 500 series revolutionizes liquid transfer by using acoustic energy to eject fluids. The Echo 500 series allows for assay miniaturization to previously unattainable volumes. Echo liquid handlers transfer 2.5 nL droplets repeatedly, so precision and accuracy are consistent over a larger volume range. Large volume transfer is achieved by transferring several droplets per second. Transfer is non-contact and tipless, with increased cost savings from elimination of tip costs and washing fluids. Miniaturization with the Echo liquid handler retails high assay performance, allowing quantitative results at higher assay well densities. The Echo liquid handler can be used to transfer from any source well position to any destination well position. These can be simple fluids (media for growing cells, buffers, DMSC) or viscous solutions (lys buffers, antibodies with glycerol, or transfection reagents).

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of drug candidates have been widely adopted as an integral part of modern drug discovery, for compound advancement and clinical candidate selection. They are critical factors in predicting pharmacokinetic behavior and drug-drug interaction potentials. One of the most important ADMET assays is inhibition of cytochrome P450 enzymes. It is inevitable that a large number of compounds will be screened against CYP enzymes. The ability of miniaturization of ADMET assays using the Echo liquid handler can be a valuable tool in drug discovery research.

P450-Glo™ Screening Assay

A fast, sensitive and easy to use assay to measure P450 activity

CYP3A4 activity in Human Liver Microsomes using P450-Glo™ screening system

The purpose of this experiment was to evaluate the feasibility using HLM and Luciferin-IPA in a low volume 384-well format.

Methods

Pooled HLM (Catalysis, CIT) at 20 ng/mL was diluted to 0.1 and 0.2 mg/mL in potassium phosphate buffer. Luciferin-IPA was also diluted in potassium phosphate buffer and NADPH regeneration system to 16 µM. 5 µL of HLM at 0.1 and 0.2 mg/mL was first added manually into a 96-well black scotter plate at a volume of 5 µL. The reaction was initiated by the addition of 5 µL diluted Luciferin-IPA for both experiments. The P450 reaction was incubated for 10 minutes at 37°C. At 10 µL of P450-Glo detection reagent was added to the mixture. Light output was monitored on the GloMax® Multi+ luminometer.

CYP3A4 activity comparison between Echo transfer and manual transfer

To test the ability of the Echo liquid handler to directly transfer HLM into a 384-well plate.

Methods

Pooled HLM (Catalysis, CIT) at 20 ng/mL was diluted to 5 mg/mL in potassium phosphate buffer. Luciferin-IPA was also diluted in potassium phosphate buffer and NADPH regeneration system. 45 µL HLM at 5 mg/mL was first added to a 384-well polypropylene Echo qualified source plate. 55 µL of HLM was then transferred from the source plate by the Echo 555 into a 384-well black scotter plate containing 5 µL potassium phosphate buffer. The reaction was initiated by the addition of 5 µL diluted Luciferin-IPA for both experiments. The P450 reaction was incubated for 10 minutes at 37°C. At the end of incubation, 10 µL P450-Glo detection reagent was added to the mixture. Light output was monitored on the GloMax® Multi+ luminometer.

Miniaturization of P450-Glo™ assay

Goal: to develop a miniaturized P450 assay with HLM in a 384-well plate.

Methods

Pooled HLM was diluted in potassium phosphate buffer to 1 mg/mL. Luciferin-IPA was diluted in both potassium phosphate buffer and NADPH regeneration system to 10 µM. 40 µL HLM and 40 µL Luciferin-IPA in either potassium phosphate buffer and NADPH were added to a 384-well plate containing Echo qualified scotter plate. 95 µL of buffer was then transferred by the Echo 555 into the scotter plate containing 5 µL potassium phosphate buffer with the Echo 555. The reaction was incubated for 10 minutes at 37°C, then 2 µL P450-Glo detection reagent was added to the reaction. Light output was monitored with the GloMax® Multi+ luminometer.

Results

The P450-Glo™ assay works properly in a small scale format in 384-well plate. This HLM showed sensitive and NADPH dependent CYP3A4 activity, making it a suitable target in the miniaturized assay format.

CYP compounds profiling in the miniaturizing assay format

To validate the miniaturized P450-Glo™ assay, IC₅₀ curves were generated for four control compounds. The IC₅₀ concentrations generated by the Echo liquid handler were compared to previously published IC₅₀ concentrations.

Methods

Control compounds were first dissolved in 100% DMSO and transferred into a 384-well, polypropylene Echo qualified plate. Three intermediate concentrations were generated using Echo® Dose Reagent software. 1:10 of each compound from the source plate was transferred to the 384-well scotter plate using the Echo 555. The IC₅₀ of each compound was transferred into the scotter plate containing Echo qualified scotter plate. The reaction was initiated by the addition of 5 µL diluted Luciferin-IPA for both experiments. The P450 reaction was incubated for 10 minutes at 37°C. At the end of incubation, 10 µL P450-Glo detection reagent was added to the mixture. Light output was monitored with the GloMax® Multi+ luminometer.

Results

The data showed the miniaturized P450-Glo™ assay provided inhibition data that were consistent with published literature values.

Summary

• Successfully miniaturized the P450-Glo™ assay to a 1 µL total reaction volume in a 384-well format.
• Transferred HLM with the Echo liquid handler.
• A miniaturized P450-Glo™ assay has increased utility in screening ADMET profiles in an earlier stage in the drug discovery process.
• Compounds and other reagents can be transferred efficiently using Echo® applications software.