



Screen More With Less

Ultrahigh-Throughput Compound Profiling Using Next-Generation Dispensing Technology

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Introduction

The ability to better understand the properties of individual compounds is essential in the drug-discovery process. With compound libraries many times numbering in the millions, screening facilities have begun incorporating high-density 1536-well plates in a miniaturized format as a streamlined, cost-effective approach for screening large compound libraries. For this approach to work, they need easy-to-perform assays that are sensitive in a low-volume format. In addition, they need a way to accurately dispense assay components at these volumes.

By miniaturizing a process into high-density 1536-well plates, more meaningful data can be generated by analyzing the effect of a wide range of compound concentrations.

To save time and money, researchers have begun generating compound profiles. Target-based and ADME/Tox assays are run earlier in the discovery process to better predict off-target activity and toxicity. As large numbers of similarly structured compounds are taken through the same profiling process, it becomes easier to predict the effects of each compound class. Performing the assays using high-density plates also allows the screener to incorporate a dilution series of each test compound. This enables the researcher to better understand how a compound will affect a certain target over a wide concentration range. In this application we demonstrate this approach using Promega luminescent and fluorescent HTS assays for profiling test compounds in a miniaturized ultrahigh-throughput setting.

Luminescent and Fluorescent High-Throughput Assays

We chose four compounds from two distinct compound classes to determine the effects that each had on a varied group of target-focused and ADME/Tox assays. The first two, nifedipine and nifedipine, are calcium channel blockers, while the second two, dexamethasone and progesterone, are steroids. Each compound was tested at concentrations ranging from 0.1 μ M–100 μ M to generate IC₅₀ or EC₅₀ values for each compound/chemistry combination. All assays were run in 1536-well format (Table 1).

For the Dual-Glo™ GPCR assay, we plated stably transfected HEK 293 cells in a single 1536-well plate at a density of 2,500 cells/well and added compounds to all wells for the agonist and antagonist assays. SKF38393 was added to antagonist assay wells 15 minutes later, and the plate was incubated for 4 hours at 37°C. Dual-Glo™ Luciferase Reagent was added, and the plate was incubated for 10 minutes before recording firefly luciferase relative light units (RLU). Finally, we added the Dual-Glo™ Stop & Glo® Reagent and recorded the *Renilla* RLU following a similar incubation.

For the cell-based assays, we plated Jurkat cells on a single 1536-well plate at a density of 2,500 cells/well. Compounds were added for either 4 or 18 hours at concentrations ranging from 0–100 μ M. After the assay reagents were added, the plate was incubated for 60 minutes, and the RFU or RLU were recorded.

Table 1. Volumes Dispensed by the BioRAPTR FRD® Workstation. For the CytoTox-ONE™ chemistry, the 4 μ l detection reagent addition is followed 10 minutes later by a 2 μ l stop reagent addition to stabilize the fluorescent signal. For the Dual-Glo™ chemistry, Dual-Glo™ Luciferase Reagent is added, and the firefly luminescence is measured. Stop & Glo® Reagent is then added, and *Renilla* luminescence is measured.

| | Apo-ONE® Assay | CytoTox-ONE™ Assay | CellTiter-Glo® Assay | Caspase-Glo® 3/7 Assay | Dual-Glo™ GPCR Assay | P450-Glo™ Assay | Kinase-Glo® Plus Assay |
|----------------------|----------------|---------------------|----------------------|------------------------|-------------------------|-----------------|------------------------|
| Cells | 2 μ l | 2 μ l | 2 μ l | 2 μ l | 1 μ l | — | — |
| Compound Addition | 2 μ l | 2 μ l | 2 μ l | 2 μ l | 2 μ l/0.5 μ l | 2 μ l | 2 μ l |
| Enzyme/Substrate Mix | — | — | — | — | — | 2 μ l | 2 μ l |
| NADPH/ATP | — | — | — | — | — | 1 μ l | 1 μ l |
| Detection Reagent | 4 μ l | 4 μ l/2 μ l | 4 μ l | 4 μ l | 3.5 μ l/3.5 μ l | 5 μ l | 5 μ l |

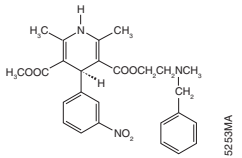
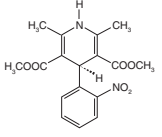
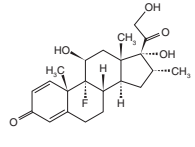
Ultrahigh-Throughput Profiling... continued

For the biochemical assays, we dispensed compounds into a single 1536-well plate at concentrations ranging from 0–100µM followed by enzyme/substrate mix addition. Next we added NADPH (P450-Glo™ Assay) or ATP (Kinase-Glo® Plus Assay) and incubated at room temperature for the appropriate time. Finally, the appropriate detection reagent was added, the plate was incubated according to the directions in the technical bulletin for each chemistry, and the RLU were recorded.

Non-Contact Dispensing Instrumentation

The Aurora Discovery BioRAPTR FRD® Workstation was used for dispensing cells, compounds, ATP, enzymes/substrates and reagents into the 1536-well microplates. The BioRAPTR FRD™ is representative of a unique class of automated liquid handlers that incorporate rapid, on-the-fly, non-contact dispensing technologies to distribute liquids into 96-, 384-, 1536-, and 3456-well plates. Typically this type of technology can dispense volumes ranging from 60µl down to 100nl.

Table 2. Data from Individual Miniaturized Profiling Assays. Results are expressed in µM concentrations unless otherwise noted.

| |  8253MA |  5254MA |  5256MA |  5255MA |
|--|---|---|--|---|
| | Nicardipine | Nifedipine | Progesterone | Dexamethasone |
| Kinase-Glo® Plus Assay PKA Assay | 0.075µM | 0.094µM | >100µM | >100µM |
| Dual-Glo™ GPCR Assay Agonist Assay | No Induction | No Induction | No Induction | No Induction |
| Antagonist Assay | No Inhibition | No Inhibition | No Inhibition | No Inhibition |
| Apo-ONE® Assay 4-Hour Incubation | No Effect | No Effect | >100µM | >100µM |
| 18-Hour Incubation | >10µM | >10µM | >10µM | >10µM |
| CytoTox-ONE™ Assay 4-Hour Incubation | No Effect | No Effect | No Effect | No Effect |
| 18-Hour Incubation | No Effect | No Effect | No Effect | No Effect |
| CellTiter-Glo® Assay 4-Hour Incubation | Toxic >10µM | Non-Toxic | Non-Toxic | Non-Toxic |
| 18-Hour Incubation | Toxic >10µM | Toxic >50µM | Toxic >50µM | Toxic >50µM |
| Caspase-Glo® 3/7 Assay 4-Hour incubation | 5.728µM | 2.388µM | 5.133µM | 5.653µM |
| 18-Hour Incubation | 0.1513µM | 0.0939µM | 0.3237µM | 0.0986µM |
| P450-Glo™ Assay CYP1A2 Assay | 14.14µM | 1.2µM | No Inhibition | 10.6µM |
| CYP2C9 Assay | 0.06µM | 0.3µM | 0.32µM | 1.0µM |
| CYP3A4 Assay | 1.1µM | 2.1µM | No Inhibition | >100µM |
| CYP2C19 Assay | 2.3µM | 2.5µM | 5.1µM | >100µM |
| CYP2D6 Assay | 6.2µM | 8.5µM | >100µM | 53.6µM |

Inhibitory/Toxic Effects <10µM

Non-inhibitory/Non-toxic Effects

For this application, the dispensed volumes ranged from 5µl to 500nl. Table 1 shows the exact volumes that were added to the assay plate for each chemistry.

By performing this application in 1536-well format, we had the unique advantage of being able to profile all four compounds with multiple chemistries on the same assay plate. This includes combining assays for five CYP450 isoforms, as well as the combination of Apo-ONE®, CytoTox-ONE™, CellTiter-Glo®, and Caspase-Glo® 3/7 Assays. Figure 1 shows an example of how this was accomplished.

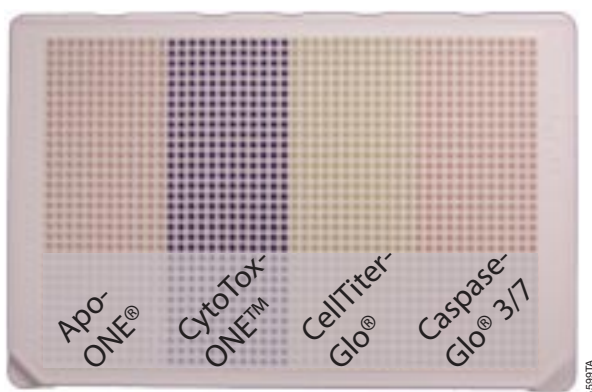


Figure 1. Layout of 1536-well plate. Example of how compounds are profiled using multiple chemistries in a 1536-well plate.

Results

Using these profiling assays, we generated IC₅₀ or EC₅₀ values to ascertain the effects that each compound had upon the various assay chemistries (Table 2). We categorized the results into two distinct groups to better visualize any trends within and between the compound classes. The first group showed strong inhibitory or toxic effects, which we determined to be below a 10µM concentration. The second group showed weak or no inhibitory effects. We then gave each group a color coding in the table, red for strong effects, and green for weak or no effects (Table 2). By using this color scheme, it became apparent that the compounds within each class have almost the same effect on all of the assays included in the profile. Also, there are distinct differences in the effects between the compound classes.

Discussion

As the data illustrate, running profile screens including both target-focused and ADME/Tox assays can provide a wealth of information about test compounds earlier in the screening process. Each individual profile can assist in deciding whether or not to move forward with a compound, saving both time and money. As large numbers of similarly structured compounds are taken through the same profile process, it becomes easier to predict the effects of each compound class. By

miniaturizing this process into high-density, 1536-well plates, more meaningful data can be generated by analyzing the effect of a wide range of compound concentrations. In addition, multiple assays can be run in a single plate. This further increases the amount of information coming from each screen. The combination of Promega homogeneous HTS assays and next-generation low-volume liquid dispensers provides an ideal solution to make ultrahigh-throughput compound profiling a reality.



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Ordering Information

| Product | Size | Cat.# |
|---|-----------------|-------|
| Kinase-Glo® Plus Luminescent Kinase Assay | 10ml* | V3771 |
| Dual-Glo™ Luciferase Assay System | 10ml* | E2920 |
| Apo-ONE® Homogeneous Caspase-3/7 Assay | 1ml* | G7792 |
| CytoTox-ONE™ Homogeneous Membrane Integrity Assay | 200–800 assays* | G7890 |
| CellTiter-Glo® Luminescent Cell Viability Assay | 10ml* | G7570 |
| Caspase-Glo® 3/7 Assay† | 2.5ml* | G8090 |
| P450-Glo™ CYP1A2 Assay | 10ml* | V8771 |

† For Laboratory Use

* Other sizes available.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

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