Miniaturized GPCR Signaling Studies in 1536-well Format

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Abstract

G protein-coupled receptors (GPCRs) are involved in various physiological processes such as the regulations of behavioral mode and immune system activity. GPCRs are popular targets in drug discovery, and a well-designed assay can speed up the discovery of novel drug candidates. The Promega cAMP-Glo™ Assay monitors cAMP production in cells in response to the effect of an agonist or test compound on GPCRs. The assay is based on the principle that cyclic AMP (cAMP) stimulates protein kinase A (PKA) holoenzyme activity, decreasing available ATP and leading to decreased light production in a luciferase reaction. Together with the Labcyte® Echoc™ 555 acoustic liquid handler and the Deerac Fluidics™ Equator™ HTS reagent dispenser, compounds can be screened in 1536-well format for effects on GPCRs. Implementing a high-throughput miniaturized GPCR assay as demonstrated in this poster allows cost-effective screening to identify lead modulators of GPCR signaling.

Promega cAMP-Glo™ Assay

The Promega cAMP-Glo™ Assay is a homogeneous, bioluminescent and high-throughput assay to measure cAMP levels in cells. The assay monitors cAMP production in cells in response to the effect of an agonist or test compound on GPCRs. GPCRs that couple with adenylyl cyclase will increase or decrease intracellular cAMP. The assay protocol is quick and simple, as described below:

- **A沐P®J Glo™ Lysis**: Buffer is added to the initial cells and allowed to incubate for 30 minutes at room temperature.
- **A沐P®J Glo™ Detection Solution**: A沐P®J Glo™ Detection Solution is added to the lysed cells and allowed to incubate. The 10 µL detection solution per well is incubated at room temperature.
- **Kinase-Glo™ Reagent**: Kinase-Glo™ Reagent is added to the mixture and allowed to incubate. A沐P®J Glo™ Detection Solution and 4 µL of Kinase-Glo™ were added per well sequentially.

The plate is read on a luminometer.

Deerac Fluidics™ Equator™ HTS

Deerac Fluidics™ Equator™ HTS reagent dispenser uses an eight-channel pipetting system to accurately dispense liquid volumes ranging from 50 nL to 50 µL. For this assay, 990 nL cells and various volumes (1-4 µL) of cAMP-Glo™ reagents are accurately dispensed using a stepped pipetting option. Reagents are washed thoroughly from the system between dispensings via the Active Wash Station.

Advantages to using the Equator™ HTS for dispensing reagents for this study include:

- Speed and accuracy
- Low dead volumes
- Ease of use
- Independent channel control for dispensing gradients and regions
- Intermittent-deck components for flexibility, including small or large volume capacity reservoirs, stirring devices and multiple wash stations
- Custom configurations

Luminescent assays can be performed on a single 1536-well plate, with only one endpoint readout needed to capture data. Performing a HTS screen on a single plate allows one to study hundreds of test compounds simultaneously, simplifying data analysis and sample tracking.

Implementing this high-throughput miniaturized GPCR assay using the tools demonstrated in this poster allows cost-effective screening to identify lead modulators of GPCR signaling.

<table>
<thead>
<tr>
<th>LOPAC&lt;sup&gt;1280&lt;/sup&gt; Screening Results</th>
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<tbody>
<tr>
<td>Dopamine Receptor Agonist Screen</td>
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<tr>
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<td>2</td>
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Table 1. A table highlighting the “hits” noted in Figure 8 characterized by compound class, percentage of the total hits (%), compound action, and compound selectivity.

Summary

- A well-suited platform for HTS drug and inhibitor screening
- Easy-to-follow protocol and user-friendly instrumentation
- Accurate and precise liquid handling yield reliable assay results
- Quick, homogeneous, sensitive, and non-radioactive assay
- Robust, stable reagents and detection signal

The combination of acoustic dispensing with the Echoc™ 555, small-volume pipetting with the Equator™ HTS, and robust cAMP-Glo™ Assay makes rapid screening in 1536-well format a reality.