Live-Cell Biosensor Assay Used To Interrogate GPCRs: Comparative Analysis of Biosensor Variants

P. Brescia1, P. Banks1, B. Binkowski2, P. Stecha2, N. Cosby2

1BioTek Instruments, Inc., Winooski, Vermont, USA  2Promega Corporation, Madison, Wisconsin, USA

Abstract

Functional, cell-based assays are essential to identify modulators of GPCRs, including those that signal via increases or decreases in cAMP. Previously, we demonstrated the automation of the GloSensor™ cAMP Assay from Promega, which utilizes a live-cell biosensor consisting of a fusion of a cAMP binding domain to a circularly permuted form of firefly luciferase. Upon binding to cAMP, conformational changes in the expressed sensor lead to increases in light output in the presence of substrate. Various schemes of the biosensor exist with different affinities for cAMP, some offering increased sensitivity and others offering increased dynamic range. The sensor with the broadest dynamic range can be used to screen at lowest concentrations when the broad-spectrum parameters are necessary. In this study, several variants of the GloSensor cAMP biosensor were used to identify a new variant that is highly sensitive and has an increased dynamic range.

Introduction

G-protein coupled receptors (GPCRs) primarily function as sensors of the extracellular environment and communicators of changes in that environment to intracellular machinery. The transmembrane nature of GPCRs ensures that signaling compounds, often in the form of small molecules, are capable of modulating their intracellular activity dependent on reversibly interacting with ligand binding domains in the extracellular, and not the intracellular, face of the receptor. Upon binding to a ligand, the receptor transduces the signal to the cytoplasmic G-protein located on the cytosolic face of the receptor in the cell membrane (Figure 1). Considerable effort remains focused on measuring the functional responses of the receptor to chemical compounds and determining the pharmacology of agonists and antagonists. The advent of molecular biology and the availability of these new technologies have allowed the development of many different cell lines that can be used for drug screening and drug discovery.

Cell Culture Procedure

Cells propagated using typical 96-well plate format

Figure 6 – Cell culture conditions for GloSensor cAMP HEK293 cell lines. GloSensor cAMP HEK293-20F and -22F cell lines were grown in culture to >90% confluency as per manufacturers technical manual in DMEM media supplemented with 10% FBS and Hygromycin B.

β2 Adrenergic Receptor Agonist EC50

<table>
<thead>
<tr>
<th>Compound</th>
<th>20F (nM)</th>
<th>22F (nM)</th>
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</thead>
<tbody>
<tr>
<td>Formoterol</td>
<td>0.1</td>
<td>0.43</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>6.2</td>
<td>32</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>4.8</td>
<td>19</td>
</tr>
<tr>
<td>Forskolin</td>
<td>103</td>
<td>704</td>
</tr>
</tbody>
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Table 1 – EC50 concentration of agonists as determined from dose response curves in Figure 8.

Fold Induction Calculation

- Pre-read data was used to normalize well-to-well variation due to plating variability and edge effects.
- Post incubation time data points were divided by the pre-read value for each well.
- Calculate data point ratio for each condition were then averaged.
- Fold response was calculated by dividing the signal from wells containing compound by the signal from wells containing no compound (basal signal).

β2 Adrenergic Receptor Antagonist Response

Cells were prepared and plated as described previously at a density of 2,000 cells/well

Plates were incubated overnight at 37°C, 5-10% CO2, and GloSensor cAMP Reagent added as described previously.

The plate was pre-read as described previously and antagonist added.

Figure 9 – Comparison of changes in A) luminescence signal and B) fold response of GloSensor cAMP HEK293 cell lines when subject to 10 µM ICI 118,551 antagonist and the corresponding EC50 concentration of the agonist formoterol for each variant.

The 22F variant shows lower induced luminescent intensity upon agonist addition as well as suppression of basal activity by addition of antagonist resulting in a significant increase in observed fold induction compared to the 20F variant when the assay is performed at room temperature.

β2 Adrenergic Receptor Antagonist Response

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Table 2 – Comparison of antagonist IC50 concentrations calculated from dose response curve.

Conclusions

1. The GloSensor cAMP Assay can be performed using simple, inexpensive automated methods.
2. The highly sensitive bottom-reading capabilities of the Syndrome NED HTS Multi-mode Microplate Reader provided the ability to read both the low basal signal for the lowest affinity 22F biosensor variant as well as discriminate changes from the more sensitive, reduced dynamic range 20F variant.
3. A significant increase in signal-to-background was seen in the 22F variant when compared to the 20F variant following activation of an endogenous Gs-coupled receptor in HEK293 cells when assayed at room temperature.
4. The improved S/B ratio seen when using the 22F variant allowed for the use of a 384-well low-volume, black, clear bottom microplate plate suitable for HTS applications and imaging.