Measuring Luminescence using the GloSensor™ cAMP Assay with the GloMax® Discover System

Promega Corporation

Materials Required

- GloSensor™ cAMP Assay (Cat.# E1171, E1261, E1290, E1291 and E2301)
- GloMax® Discover System (Cat.# GM3000)
- Tissue culture-treated, solid white, 96-well assay plate (Costar Cat.# 3917)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *GloMax® Discover System Technical Manual* #TM397 and *GloSensor™ cAMP Assay Technical Manual* #TM076 are available at: **www.promega.com/protocols/**

G-protein coupled, seven-transmembrane (7-TM) receptors (GPCRs) represent a major class of drug targets, and the prevalence of these receptors in physiologically important signaling events is well known. The GloMax® Discover System in combination with the GloSensor™ cAMP Assay provides an extremely sensitive and easy-to-use format for the interrogation of overexpressed or endogenous GPCRs that signal via changes in the intracellular concentration of cAMP. cAMP is detected by the use of luminescence quantitation.

The GloSensor™ cAMP Assay uses genetically encoded biosensor variants with cAMP binding domains fused to mutant forms of *Photinus pyralis* luciferase. Upon binding to cAMP, conformational changes occur that promote large increases in light output. Following pre-equilibration with substrate, cells transiently or stably expressing a biosensor variant can be used to assay GPCR function using a live-cell, nonlytic assay format, enabling facile kinetic measurements of cAMP accumulation or turnover in living cells. Moreover, the assay offers a broad dynamic range, showing up to 500-fold changes in light output. Extreme sensitivity allows detection of G_i-coupled receptor activation or inverse agonist activity in the absence of artificial stimulation by compounds such as forskolin.

The GloSensor™ cAMP Assay is made easy on the GloMax® Discover System. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allow the user to easily measure various sample signal intensities on the same plate. Using the GloMax® Discover System, data is collected using a standard 96-well plate and luminescence light output is proportional to cAMP concentration (Figure 1). This Application Note describes the protocol for measuring luminescence using the GloMax® Discover System with the GloSensor™ cAMP Assay.

Protocol

For detailed instructions and assay notes, follow the procedure as indicated in the *GloSensor™ cAMP Assay Technical Manual,* #TM076. The technical manual contains instructions for transient transfection of adherent or suspension cells with pGloSensor™ cAMP Plasmid, or for use of the GloSensor™ cAMP HEK293 Cell Line. The assay can be performed in a

96-well format as either an end-point or kinetic assay.

Sample Assay with CHO Cells

Follow sample protocol (Section 4.B) in Technical Manual #TM076 to transfect cells using FuGENE® HD transfection reagent. After a final incubation for 20–24 hours in a 37°C tissue culture incubator with 5–10% CO_2, proceed to the assay protocol.

Equilibration with GloSensor™ cAMP Reagent

1. Carefully remove the medium from the individual wells. To accomplish this, place the pipette tips at the side of the well to minimize disruption of the cell monolayer. Move quickly to Step 2.
2. Add 100µl of equilibration medium per well for a 96-well plate. Add medium to the side of each well; do not pipet directly onto the cell monolayer. The equilibration medium contains a 2% v/v dilution of the GloSensor™ cAMP Reagent stock solution.
3. Incubate for 2 hours at room temperature or until a steady-state basal signal is obtained. Incubation at higher temperatures can facilitate equilibration, but care must be taken to allow the entire plate to come to a uniform temperature prior to starting the assay.

Compound Preparation

To obtain a concentration response curve, serially dilute the compound in storage solvent (aqueous solution or DMSO) to 100X stock solutions, followed by direct addition to the respective wells. Alternatively, serially dilute the compound in storage solvent to 1,000X stock solutions, followed by dilution to 10X aqueous stock solutions and delivery to the respective wells.

End-Point Analysis at Room Temperature

1. Take a pre-read measurement prior to compound addition. Although this step is not required, normalization of data to a pre-read measurement can increase data quality by removing the well-to-well variability associated with transient transfection and differing total cell numbers.
2. Add 1µl of 100X compound stock solution or 10µl of a 10X compound stock solution per well using a multichannel pipet. Gently mix without disturbing the cell monolayer. We have found no deleterious effects associated with running assays using a 1% final DMSO concentration.
3. Measure luminescence. See Section 3.A for recommended times for measurement after compound addition, depending on assay format.

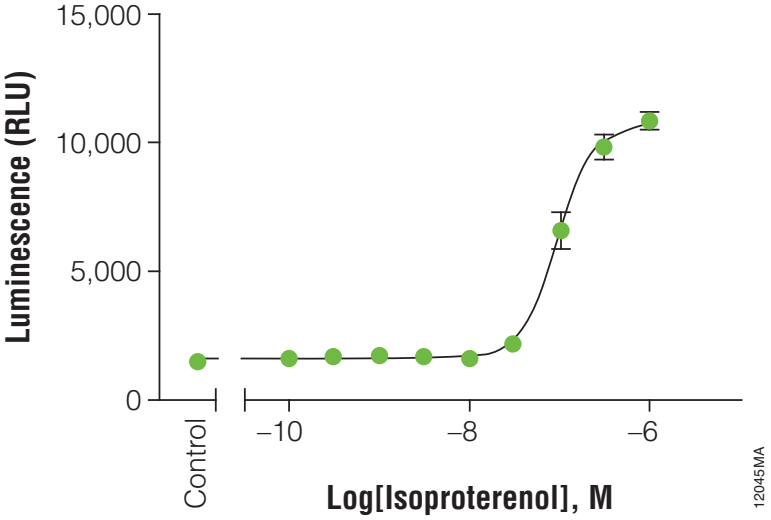

Figure 1. Performance of GloSensor™ cAMP Assay with HEK293 cells following activation of an endogenous G_s-coupled, 7-TM receptor. HEK293 cells were transiently transfected with pGloSensor™-22F cAMP Plasmid and assayed following the protocol outlined in Technical Manual #TM076, Section 4.B. Luminescence was measured 10 minutes after addition of varying concentrations of isoproterenol, a full β2-adrenergic receptor agonist. Each point represents the average and standard deviation of four replicates.

GloMax® Discover System

The GloMax® Discover System offers superior sensitivity, dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System also provides flexible use of filters for fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance measurements for adaptation into a wide variety of laboratory applications. The instrument is operated by an integrated tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting to your local network. .