Measuring cAMP Levels Using the cAMP-Glo™ Assay and GloMax® Discover System

Promega Corporation

Materials Required

- cAMP-Glo™ Assay (Cat.# V1501, V1502 and V1503)
- GloMax® Discover System (Cat.# GM3000)
- Induction buffer [Krebs Ringer buffer, 1X phosphate-buffered saline (PBS) or serum-free medium containing 500µM isobutyl-1-methylxanthine (IBMX, Sigma-Aldrich Cat.# I7018) and 100µM 4-(3-butoxy-4-methoxy-benzyl) imidazolidone (Ro 20-1724, Sigma Aldrich Cat.# B8279)], at room temperature
- White, clear-bottom tissue culture plates

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 *cAMP-Glo™ Assay Technical Bulletin* #TB357 are available at: **www.promega.com/protocols/**

Characterization of cell signaling pathways is important for developing more effective drugs that can reverse dysregulated signaling events. Cyclic AMP (cAMP) is a key second messenger involved in G protein-coupled receptor (GPCRs) signaling and is a useful indicator of the modulation of these important signaling pathways (1). The cAMP-Glo™ Assay is a homogeneous, bioluminescent and high-throughput assay to measure cAMP levels in cells. The cAMP-Glo™ Assay monitors cAMP production in cells in response to the effects of an agonist or test compound on GPCRs. GPCRs that couple with adenylate cyclase will increase or decrease intracellular cAMP. The assay is based on the principle that cAMP stimulates protein kinase A (PKA) holoenzyme activity, decreasing available ATP. The decrease in ATP concentration can be monitored as a decrease in luminescence using luciferase in a reaction that requires ATP to produce light. Luminescence is inversely related to cAMP concentration (Figure 1) and can be correlated to cAMP concentration using a cAMP standard curve.

Performing the cAMP-Glo™ Assay is easy with the GloMax® Discover System. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allows you to easily measure signals of varying intensities on the same plate. Using the GloMax® Discover System, data are collected in a standard 96-well or 384-well plate format. This Application Note describes the protocol to measure cAMP levels using the cAMP-Glo™ Assay and GloMax® Discover System.

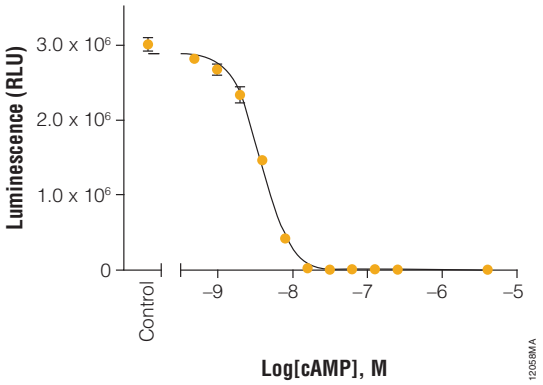

Figure 1. Titration of purified cAMP. Reactions were assembled with the indicated concentrations of purified cAMP in a 96-well plate. The cAMP-Glo™ Assay was performed as described in Section 5.A of the *cAMP-Glo™ Assay Technical Bulletin* to generate a standard curve. Data were collected using the GloMax® Discover System. Each point represents four data points; error bars represent the standard deviation. Data analysis was performed with GraphPad Prism® software, version 6.0, using a sigmoidal dose-response (variable slope) equation.

cAMP-Glo™ Assay Protocol

For detailed instructions and assay notes, see the *cAMP-Glo™ Assay Technical Bulletin* #TB357. This Application Note demonstrates that the GloMax® Discover System can detect luminescence generated using the cAMP-Glo™ Assay over the range of cAMP concentrations shown in Figure 1.

Reagent Preparation

1. Thaw all components except Protein Kinase A completely at room temperature before use. Store Protein Kinase A on ice when not at –20°C.
2. Prepare cAMP solution by adding 1µl of 1mM cAMP to 250µl of induction buffer. Vortex to mix.

   Note: For the experiments described in Figure 1, 1X PBS was used as the induction buffer. Other induction buffers can be used; see the *cAMP-Glo™ Assay Technical Bulletin* #TB357.
3. Transfer the entire volume of Kinase-Glo® Buffer to the amber bottle containing the Kinase-Glo® Substrate to form the Kinase-Glo® Reagent. Mix by gentle vortexing

Standard Curve Preparation

In a separate 96-well plate:

1. Add 100µl of induction buffer to wells A2 through A12.
2. Add 200µl of the cAMP solution prepared earlier to well A1.
3. Perform a serial twofold dilution by transferring 100µl from well A1 to well A2 in column 2, pipetting to mix. Transfer 100µl to well A3, and mix. Repeat for wells A4 through A11. Discard the extra 100µl from well A11. Do not add cAMP solution to the no-cAMP control reaction in well A12.
4. Transfer 20µl of each dilution to wells reserved for the cAMP standard curve on the assay plate (in triplicate).

Assay Protocol

1. Add 20µl of cAMP-Glo™ Lysis Buffer to all wells. Incubate plate with shaking at room temperature for 15 minutes.
2. Prepare the cAMP Detection Solution by mixing 2.5µl of Protein Kinase A and 1.0ml of cAMP-Glo™ Reaction Buffer immediately before use.
3. Add 40µl of cAMP-Glo™ Detection Solution to all wells, and mix the plate by shaking for 1 minute. Incubate the plate at room temperature for 20 minutes.
4. Add 80µl of room-temperature Kinase-Glo® Reagent to all wells. Mix the plate by shaking for 1 minute, and incubate at room temperature for 10 minutes.
5. Measure luminescence with the GloMax® Discover System.
   a. Select the door icon at the top right on the home page.
   b. Once the door opens, place the plate in the holder with well A1 of the plate at the front left corner.
   c. Select the door icon to close the door.
   d. Select “Protocols” from the home page, then select “Preset” on the left menu.
   e. Select “cAMP-Glo” from the list of protocols.
   f. To assign assay wells, select the plate icon next to the lock icon. Highlight the assay wells (green wells will be assayed; white wells will not). Select “OK”.
   g. Select “Start” to begin the protocol.
   h. Once measurements are completed select “Export”. The data will be exported as a CSV file and an Excel® file.
6. Follow the instructions in the *cAMP-Glo™ Assay Technical Bulletin* to generate the cAMP standard curve to quantify cAMP.

Conclusion

The GloMax® Discover can detect luminescence generated using the cAMP-Glo™ Assay over the range of cAMP concentrations shown in Figure 1.

The GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and 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 allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for 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 data to your local network.

Reference

1. Ellis, C. *et al.* (2004) The state of GPCR research in 2004. *Nat. Rev. Drug Discov.* **3**, 577–626.

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