

A Homogenous, High Through-Put Luminescent cAMP Assay to Monitor Modulation of Gs and Gi Protein Coupled Receptors

Said A. Goueli^{1,2}, Meera Kumar¹, Kevin Hsiao¹, Jolanta Vidugiriene¹, and Bob Bulleit¹

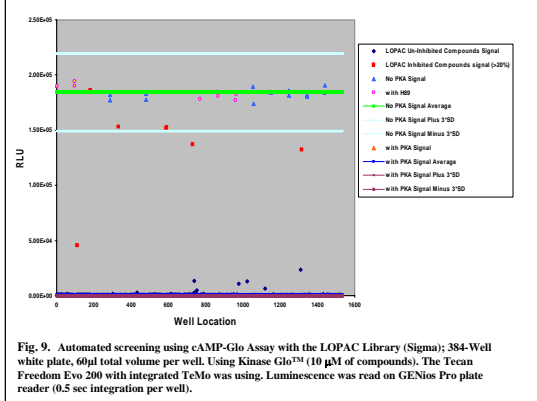
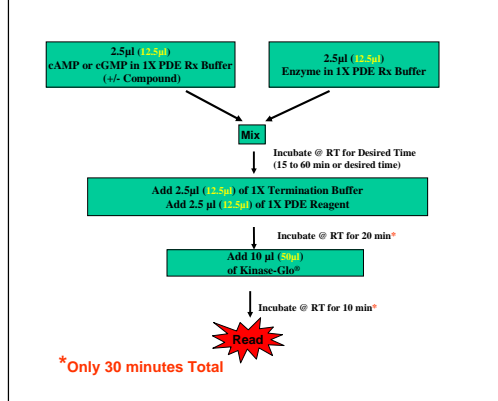
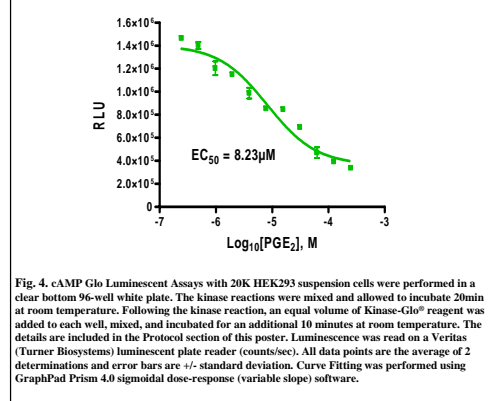
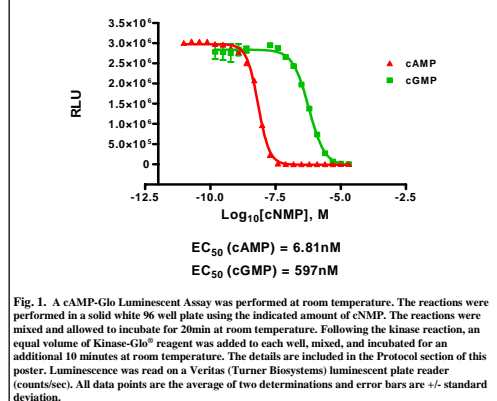
¹Cell Signaling Group, Cellular Analysis Platform, Dept. R&D, Promega Corp., and ²Dept. Of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI
Promega Corporation, 2800 Woods Hollow Road, Madison WI 53711, USA (Email: Said.Goueli@Promega.com)



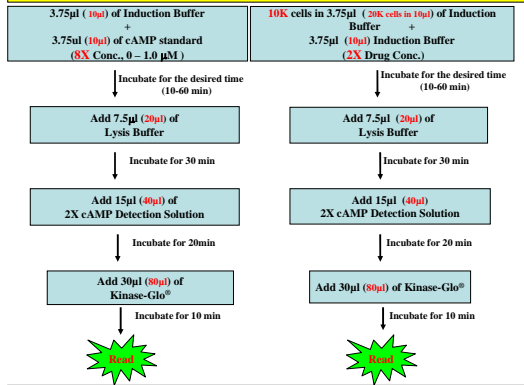
Promega

Abstract | cNMP Titration using cAMP-Glo Luminescent Assay | Titration of Endogenous Prostaglandin Receptor Using PGE₂ as Agonist in cAMP-Glo Assay | Assay Protocol for Promega PDE-Glo: 384-Well (96-well) | LOPAC Screening Using cAMP-Glo Assay (with >20% Inhibition)

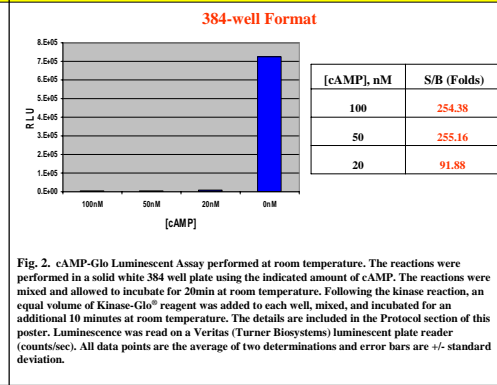
Abstract
G Protein Coupled Receptors represent a large validated target for drug discovery research. They are classified into three main groups based on the G protein associated with the receptor. The Gs group is coupled to activation of adenylate cyclase and Gi group is coupled to inhibition of adenylate cyclase while Gq is coupled to activation of phospholipase b. There are two main strategies to monitor the activation of GPCRs, reporter based assays and cAMP accumulation based assays. We report here on a new assay for monitoring modulation of GPCRs that are linked to activation or inhibition of adenylate cyclase (Gs or Gi). The assay can be used to monitor cAMP accumulation or depletion in the cell upon treatment with agonists or antagonists of Gs or Gi coupled receptors. The assay is homogenous, and amenable to high through screening of modulators of GPCRs. A Z' value higher than 0.7 attests to the robustness of the assay and is carried out in 96-, and 384-, well plates and potentially adaptable to 1536-well format. The assay is based on luminescence and thus it does not suffer from fluorescence interference by library compounds. We have successfully generated EC₅₀ values for agonists and IC₅₀ values for antagonists of Gs coupled receptors that are similar to those reported in the literature. The assay is easy to use, can be carried out in less than 60 minutes and does not require antibodies or expensive instrumentation for signal detection. The signal output is relatively stable for several hours and thus can be used for screening large numbers of plates. This luminescent assay is fast, homogenous, and reliable, as indicated by high Z' values, making it an attractive screening tool for identifying agonist or antagonists that modulate Gs and Gi coupled receptors.



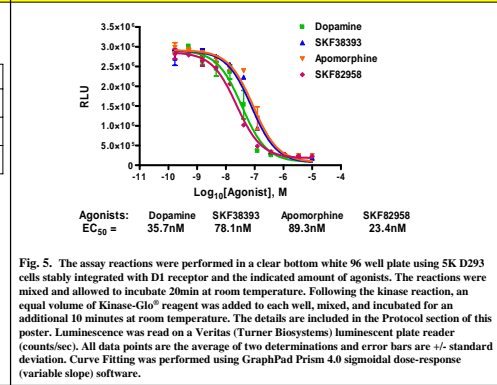
Assay Protocol for cAMP-Glo: 384-Well format (96-well): Suspension cells



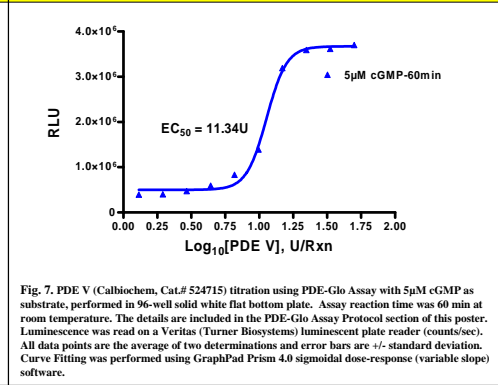
Signal:Bkgd (Ratio) at Different Concentrations of cAMP Using cAMP-Glo Assay



Agonists Titration Using Stably Integrated D1 - Receptor in D293 cells



PDE V Titration with 5µM cGMP in PDE-Glo Assay

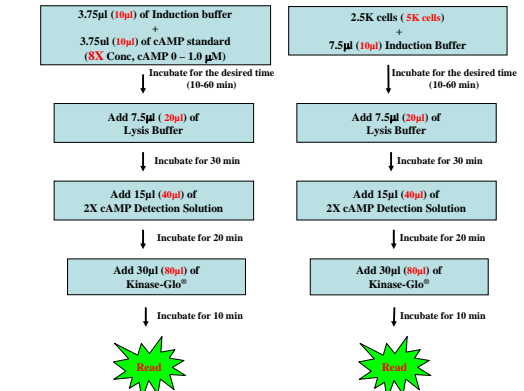


LOPAC Library Screen Results by Using cAMP Glo Luminescent Assay

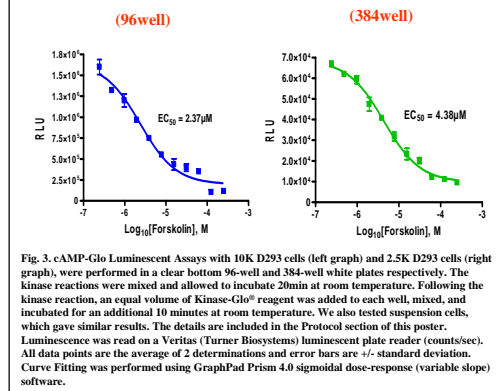
Well (Location)	Compound's Name	% Inhibition
2 - F11	H89	100
2 - H2	H9 Dihydrochloride	25
4 - D6	4-Chloro Mercuribenzoic Acide	83
7 - C2	(-) - Ephedrine Hemisulfate	82
7 - G2	Ebselen	83
8 - B8	Hydroquinone	74
14 - D9	SCH-202676 Hydrobromide	72

Table 1. The results from Automated HTS Screening of LOPAC with PKA.

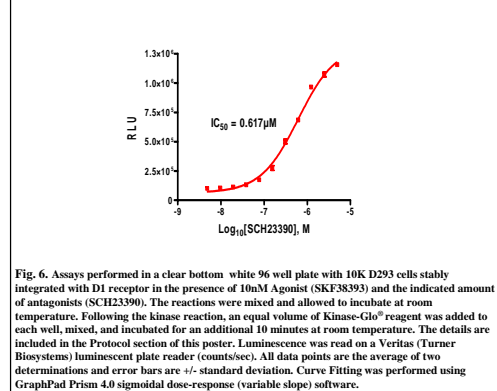
Assay Protocol for cAMP-Glo: 384-Well (96-well): Adherent cells



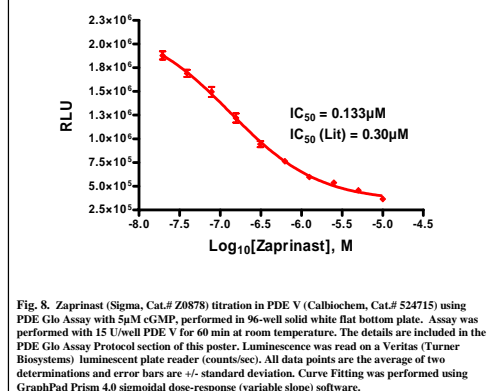
Forskolin Titration using cAMP-Glo Assay



Antagonist Titration Using Stably Integrated D1 - Receptor in D293 cells



Zaprinast Titration in PDE V with 5µM cGMP



Features of Promega cAMP-Glo™ Luminescent Assay

- Appropriated Pharmacology: Does Not Affect GPCR Pharmacology
 - Proximity to the Receptor to Minimize False Positives
 - Easy and Fast: 30min Assay, 2 Steps after Cell Lysis
 - Antibody Free: No Requirement for Antibodies
 - Optimal Platform for Screening Inhibitors
 - Suited for HTS Drug Screening: Formatted to 96-, 384-, and 1536-well plates
 - Homogeneous, Simple, Sensitive, Non-Radioactive
 - No Interference from Fluorescent Compounds, and low compound interference
 - Robust: Good Z' Values (> 0.7)
 - Stable Reagents and Detection Signal
 - Inexpensive Instrumentation Requirements
- For technical information: Said.Goueli@Promega.com