

A Homogenous, High-throughput Luminescent cAMP Assay to Monitor Modulation of G α s- and G α i- Protein Coupled Receptors

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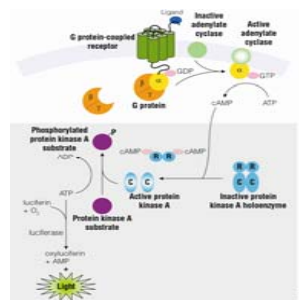
1. Abstract

Functional activities of G α s- and G α i-coupled G protein receptors can be detected by measuring changes in cAMP production. We describe the development of the cAMP-Glo™ Assay for high-throughput measurement of cAMP levels in cells.

cAMP-Glo Assay is versatile and well suited for cell-based functional GPCR receptor assays for drug screening.

- Efficiently measures both agonist and antagonist activities of Gs- and Gi-coupled receptors, and due to its high sensitivity.
- Can be used with adherent, suspension, and frozen live cells as well as membrane preparations.
- Monitor activity of GPCR receptors expressed under regulated mammalian expression system.

2. Assay Concept



cAMP-Glo Assay monitors cAMP production in cells in response to the effects of an agonist or test compound on GPCRs.

3. Assay Protocol

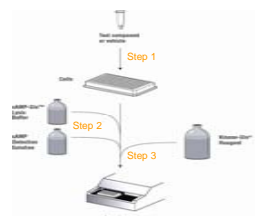
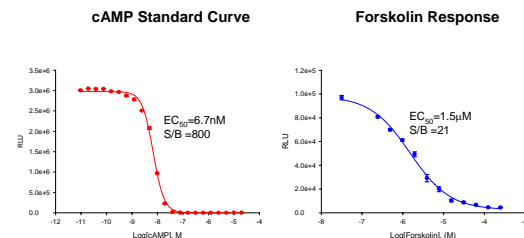


	Plate Format	Plate Format			
		96-well	384-well	384-well (LV)	1536-well
Step 1	Test compound	10µl	3.75µl	0.5µl	0.5µl
	Cells	10µl	3.75µl	0.5µl	0.5µl
Step 2	Lysis Buffer	20µl	7.5µl	1.0µl	1.0µl
	Detection Solution	40µl	15.0µl	2.0µl	2.0µl
Step 3	Kinase-Glo	80µl	30µl	4.0µl	4.0µl

4. Assay Performance



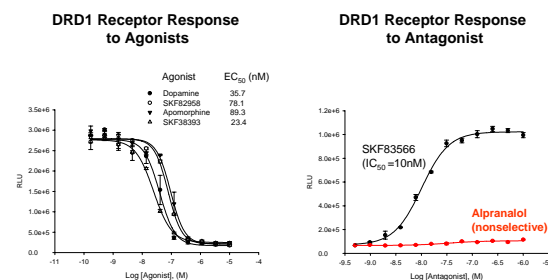
cAMP standard curve generated using cAMP-Glo assay show wide dynamic range (2×10^3 to 3×10^6 RLU), high sensitivity (lower nM range) with excellent S/B (800).

5. Robustness of cAMP-Glo Assay

Addition	Concentration	Fold Induction	Z' Value
cAMP	20nM	91	0.84
cAMP	100nM	254	0.85
Dopamine (384-well)	100nM	25.5	0.86
Dopamine (1536-well)	100nM	15	0.63
Forskolin	5µM	8	0.70
Forskolin	100µM	26.6	0.88

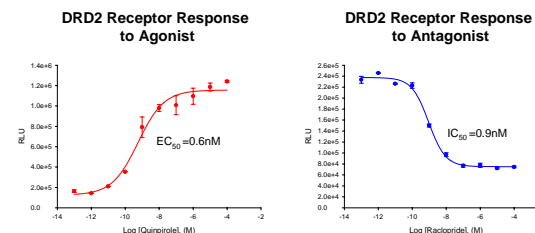
The robustness of the cAMP-Glo Assay was evaluated by calculating the Z' values for cAMP at 20 and 100nM as compared with control, and for HEK293 cells stably expressing DRD1 receptor stably expressing DRD1 receptors with dopamine (100nM) or Forskolin (5,100µM) as compared with control containing vehicle only.

6. Gas-coupled Receptor Assay



Gas agonist and antagonist assays were validated using HEK293 cells stably expressing DRD1 receptor in 384-well plates.

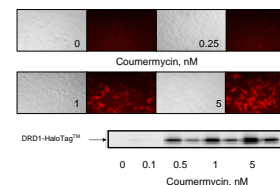
7. G α i-coupled Receptor Assay



G α i agonist and antagonist assays were validated using HEK293 cells stable expressing DRD2 receptor in 384-well plates.

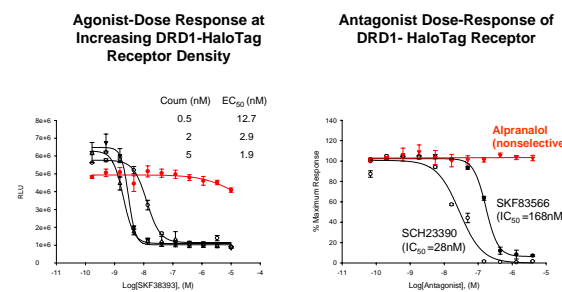
8. Regulated Expression of GPCR Receptors

Dose-dependent Expression of Dopamine (DRD1) Receptor Fused with HaloTag® Protein in a CHO Stable Cell Line



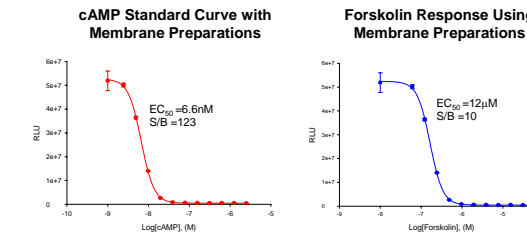
CHO cells stable expressing DRD1-HaloTag fusion receptor under coumestrolin/ novobiocin regulated expression system were exposed to increasing amounts of coumestrolin, and 24 hours after induction the cells were stained with HaloTag TMR Ligand.

9. Pharmacology of Expressed Receptors



Regulated Mammalian Expression System (C9470) allows tightly controlled expression of functional receptors.

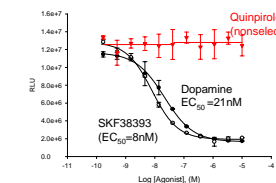
10. cAMP-Glo Assay Using Membrane Preparations



A plasma membrane preparation that maintains the integrity of GPCR/G α /AC complex was tested for monitoring cAMP concentrations upon modulation of receptors in membranes.

11. Gas-coupled Receptor Membrane-based Assay

DRD1 Receptor Agonist Pharmacology in Membranes Prepared from HEK293 Cells Stable Expressing DRD1 Receptor



Agonist dose-response on DRD1 receptor (1µg membrane/well)

A Gas agonist assay was validated on membranes prepared from HEK293 cells stable expressing dopamine DRD1 receptor.

12. Summary

cAMP-Glo Assay offers multiple features that allow researchers to easily measure cAMP accumulation in cells for GPCR studies. cAMP-Glo is bioluminescence based assay with minimal interference from fluorescent compounds and suitable for variety of instrumentation platforms.

Key Features:

- **High Sensitivity**
fmol detection limit for cAMP standard curve with S/B>500
- **Excellent Performance**
S/B>20 for Gas cell based assay
S/B>8 for G α i cell based assay
Z' > 0.8
- **Flexibility**
Whole cells or membranes
96-, 384- and 1536- well plates