HitHunter™ cAMP and cAMP Hunter™ Cell Line: Your Solution for Use with High Serum Samples, Difficult Gi Targets and Identification of Allosteric Modulators

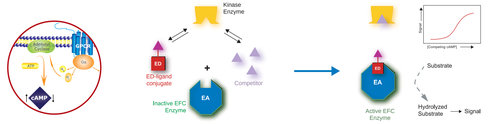
Daniel Bassoni, Mong Saetern, Rajini Bompelli, Qumber Jafri, Judy Leon, Tom Wehrman, PhD and Rueyming Loor, PhD

DiscoveRx Corporation, Fremont, CA 94538, USA

Abstract

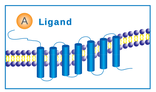
The cAMP detection technologies are widely used in high throughput screening (HTS) for measuring a functional response of a GPCR consequent to ligand-binding. However, a robust and sensitive cAMP detection platform that allows one to differentiate a full agonist from a partial agonist or an antagonist response from an inverse agonist response while also being able to identify allosteric compounds is preferred. DiscoveRx's HitHunter cAMP assay, a proven and validated platform, provides an enzyme-fragment complementation format that detects cAMP in cell lines, primary cells, neuronal cell lines, membrane preparations and in patient samples. Large assay window, high sensitivity, ability to work with very low cell number and compatibility with any standard luminometer are hallmarks of HitHunter cAMP assays. These features offer a tremendous advantage for Gi-coupled GPCRs over other available kits in the market. In this study, we demonstrate the assay compatibility with high serum levels, antibody screening and finally superiority of the platform in screening several difficult to optimize Gi cell lines. The data shows the versatility of the HitHunter cAMP assay that provides a researcher or a high throughput screener a simple one or two step chemiluminescent assay for cAMP detection.

HitHunter™ Assay Principle



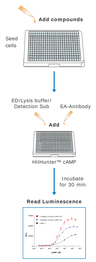
HitHunter™ cAMP assays are competitive immunoassays. Free cAMP from cell lysates competes for antibody binding against labeled cAMP (ED-cAMP conjugate). Unbound ED-cAMP is free to complement EA to form active enzyme by EFC, which subsequently hydrolyzes substrate to produce signal. A positive signal generated is directly proportional to the amount of free cAMP bound by the binding protein.

HitHunter™ and cAMP Hunter™ Work-Flow



cAMP Hunter™ cells are native Gs and Gi expressing cell lines with no-force coupling

HitHunter cAMP assays are competitive immunoassay to detect cAMP levels in cell lines, serum and primary/stem cells with low nanomolar sensitivity.



Feature	Benefit
Large assay windows	✓ Interrogate Gi- and Gs-coupled targets that do not signal effectively ✓ Ability to profile compounds with differing potencies including partial agonist
Chemiluminescent detection	✓ Minimize false positives from auto-fluorescent compounds
High Sensitivity	✓ Enables detection of cAMP in primary cells, orphans & endogenous receptors
Versatile	✓ Ideal for ANY type of compounds (agonist ✓, antagonist ✓, partial ✓, allosteric ✓, inverse ✓) ✓ Membranes, PDE inhibitors
High Serum tolerance	✓ Ideal for patient samples and antibody formulations with upto 50% serum
Excellent miniaturization	✓ 384-1536-3456 detection without compromising window and performance ✓ Minimizes assay development cost
No specialized instrument required	✓ Saves money on capital equipment ✓ Any standard reader can be used
Proven & reliable	✓ Extensively published with guaranteed performance

High Serum Applications

HitHunter cAMP can be used for

- Antibody screening
- Detecting cAMP in the presence of serum and plasma

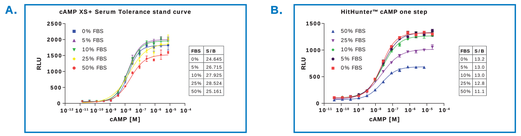


Figure 1. The cAMP standards from HitHunter cAMP XS+ **(Panel A)** and HitHunter™ one-step were diluted in various serum (FBS) concentrations and tested as per kit protocol. The data indicates that higher amounts of serum can be tolerated by the HitHunter cAMP assay. HitHunter cAMP assay is also routinely used with plasma and serum applications (Sandrock T. et.al., 2007).

Simple Chemiluminescent Detection for Agonists, Antagonists, Partial Agonists

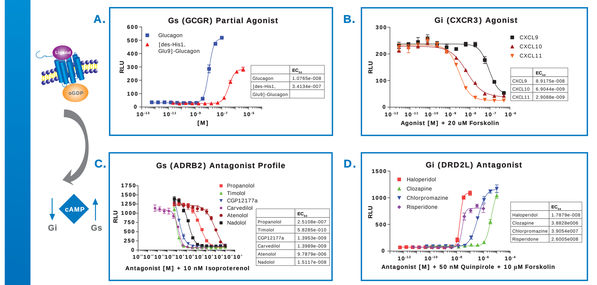


Figure 2. cAMP Hunter™ (GCGR, CXCR3, ADRB2 & DRD2L) cell lines were used in this assay. Cells were stimulated with compounds at 37°C for 30 mns in an adherent protocol. Subsequently, HitHunter™ cAMP XS+ detection reagents are added as per the kit protocol. Partial Agonist, Rank order potency of agonists, rank order of antagonists can be identified as demonstrated.

Detect Allosteric Modulators

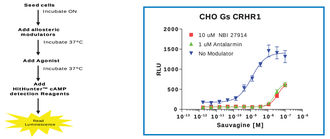


Figure 3. HitHunter cAMP assay can be used in identifying allosteric modulators (Lee et.al ;2008). The data above shows modulators of class B receptor CRHR1. cAMP Hunter™ CRHR1 cells were treated with commercially available compound NB1 27914 , Antalarmin and then agonized with Sauvagine. Compounds NB1 27914 and Antalarmin show negative modulation to Sauvagine response.

HitHunter™ cAMP to the Rescue of Difficult GPCRs

Gi Coupled CLASS A GPCRS

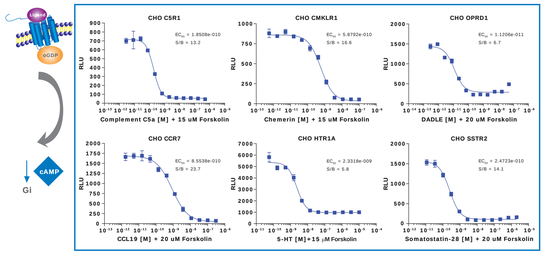


Figure 4. cAMP Hunter cells [Anaphylotoxin C5R1; chemokine receptors, CMKLR1, CCR7; Opioid receptor, OPRD1; Serotonin receptor, HTR1A & Somatostatin SSTR2] were incubated with the respective agonists, HitHunter cAMP reagents were added and plates read in any standard luminometer. Each target shown above is available as a stable cell clone. Clones are characterized for stability of 10 passages, and validated for functional cAMP response.

Gs Coupled CLASS B GPCRS

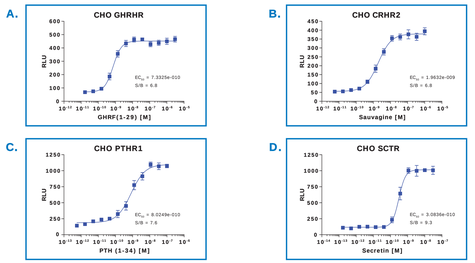


Figure 5. cAMP Hunter cells [Growth hormone-releasing hormone receptor (GHRHR), Corticotropin Releasing Factor (CRHR2), Parathyroid Hormone receptor (PTHR1) and Secretin receptor (SCTR)] were incubated with the respective agonist. cAMP response and forskolin optimization curves. HitHunter cAMP reagents were added and plates read in any standard luminometer.

Gi Coupled CLASS GPCRS

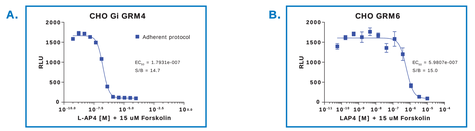


Figure 6. Class C GPCRs have a large extracellular N-terminus which binds the orthosteric (endogenous) ligand. cAMP Hunter Glutamate receptor (GRM4, GRM6) cells were incubated with the respective agonist. Each target shown above is available as a stable cell clone and is provided with functional.

HitHunter™ cAMP - an Ideal Assay Development Tool

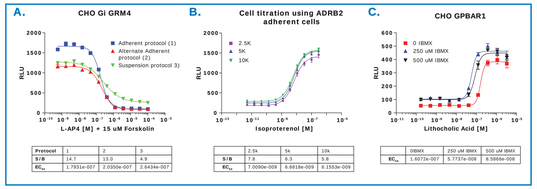


Figure 7. HitHunter cAMP assays are ideal assay development tool. **Panel A** demonstrates assay condition optimization. Results demonstrate assay plating conditions as well as adherent and suspension friendly HitHunter cAMP detection. Use of lesser cells saves tissue culture time, cost and resources. **Panel B** demonstrates cell number titration. Effect of IBMX can also be optimized for a Gs coupled receptor as shown in **Panel C**.

HitHunter™ cAMP Bibliography

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Summary

- DiscoveRx offers over 85 clonal cAMP Hunter™ cell lines validated and optimized for functional cAMP response.
- Non-force coupled cAMP Hunter cell lines exhibit robust performance and accurate pharmacology when used in conjuction with HitHunter™ cAMP assays.
- HitHunter assays have higher tolerance to serum with applicabity in screening therapeutically relevant, specific antibodies against GPCRS and also can be used in high serum applications.
- HitHunter cAMP assays provide large assay window, easy scalability and proven to be more reliable for difficult Gi targets.
- HitHunter cAMP assays allow one to screen for agonists, antagonists, partial agonists, inverse agonists and allosteric compounds.

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