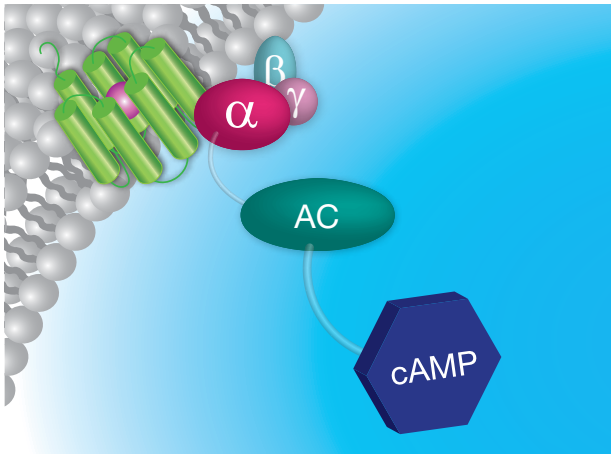
HitHunter® cAMP Assays

Optimized Assays for a Multitude of GPCR Applications

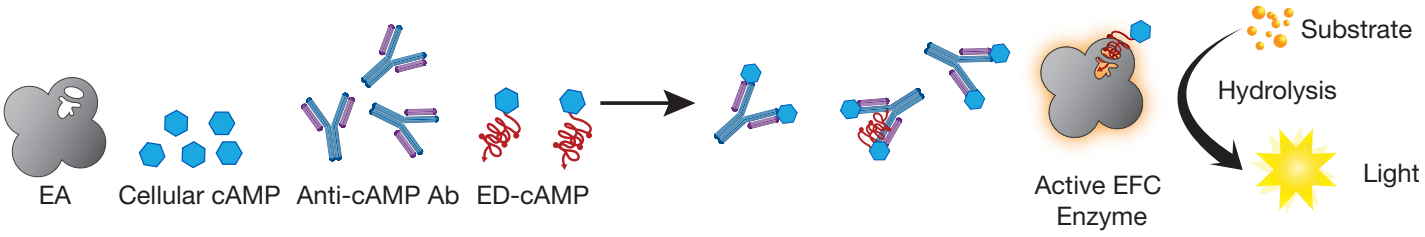
Researchers studying GPCRs need robust, easy-to-use high throughput assays that accurately detect cellular cAMP levels for a variety of ligands and applications without the need for optimization or specialized equipment. HitHunter cAMP Assays provide a simple, validated detection system, optimized with over 125 cell lines, for monitoring GPCR activation via detection of cAMP production in cells. Referenced in over 400 peer reviewed publications ranging from basic research to clinical applications, these assays provide accurate pharmacology while giving you the flexibility to work with both small molecules and biologics.



cAMP Assays Highlights

- Eliminate optimization steps with an assay that has been designed and validated for use with over 125 cAMP Hunter™ cell lines
- Achieve precise characterization of ligand pharmacology with large assay windows, sensitive detection, and wide dynamic range
- Study biologics with reproducible assay performance without fluorescence or serum interference, and no need for specialized equipment

Easy-to-Use, High Throughput Immunoassays with a Chemiluminescent Readout



HitHunter cAMP Assays are homogeneous (no wash), competitive biochemical assays that utilize DiscoverX's proprietary enzyme fragment complementation (EFC) technology to detect the level of cAMP in samples. EFC consists of a β-galactosidase (β-gal) enzyme split into two inactive components, a small enzyme donor fragment (ED) and a larger enzyme acceptor (EA). In this 2 or 3-step assay, the small ED fragment is conjugated with cAMP (ED-cAMP) while the EA fragment remains unbound. In the presence of cellular cAMP, the assay's anti-cAMP antibody becomes saturated allowing the ED-cAMP complex to complement with EA and form an active β-gal enzyme. With substrate present, the active enzyme produces a chemiluminescent signal that is directly proportional to the cellular cAMP levels and can be read on any standard luminometer.

To learn more about the HitHunter cAMP assays, visit discoverx.com/cAMP

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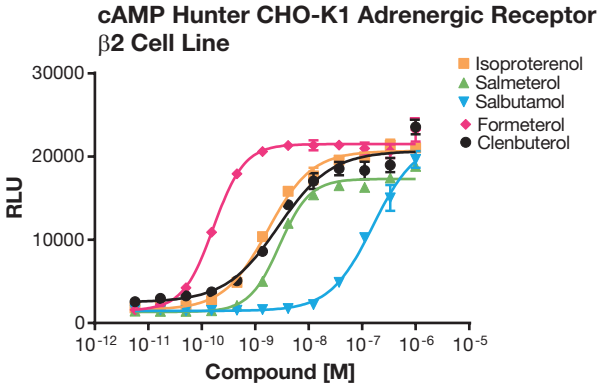
Optimized for a Variety of Applications

Accurately Rank Molecular Potency of Ligands

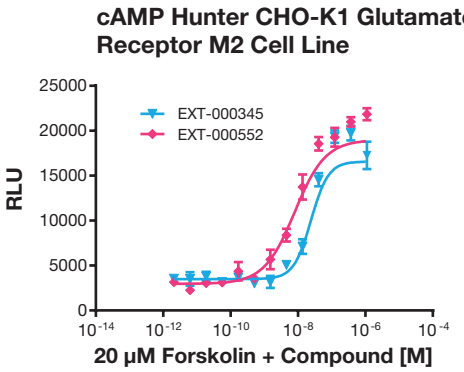
HitHunter® cAMP assays provide powerful tools for drug discovery screening and revealing of the correct ligand rank order and pharmacological profile.



Profiling experiment using a cAMP Hunter™ CHO-K1 adrenergic receptor β2 cell line to test five agonists. The data highlights the receptor's sensitivity to the various agonists, ultimately revealing the correct rank order of the agonists and showing the agonist salbutamol (EC_{50} of 160 nM) is less potent than the control ligand isoproterenol (EC_{50} of 1.7 nM).

Easily Determine Pharmacological Profiles of Complex Assay Formats

Large assay windows and sensitive detection make HitHunter cAMP assays ideal for studying complex assay formats like $G\alpha_i$-coupled receptor antagonists.

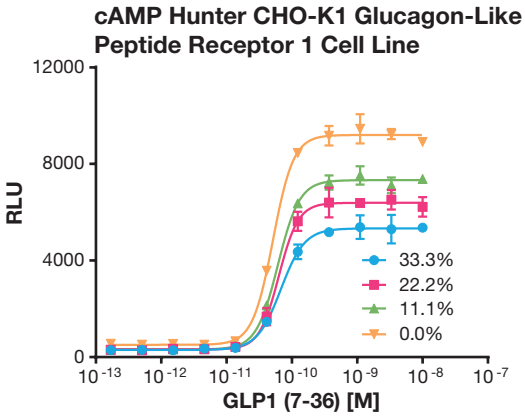


Comparative study of two antagonists of the $G\alpha_i$-coupled metabotropic glutamate receptor 2 (GRM2) using a cAMP Hunter CHO-K1 GRM2 cell line. Results indicate that the antagonist EXT-000552 (IC_{50} of 24 nM) is a more potent inhibitor compared to antagonist EXT-000345 (IC_{50} of 8 nM).

Reliable cAMP Assays for Biologics

Characterize Biologics in High Serum Samples

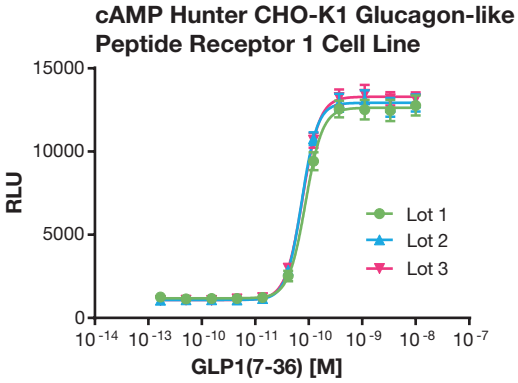
HitHunter® cAMP assays tolerate high levels of serum, which is ideal for studying biologics including immunogenicity studies to detect neutralizing antibodies.



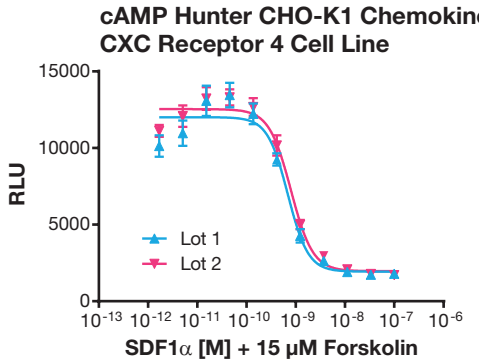
Experiment using a cAMP Hunter™ glucagon-like peptide receptor 1 (GLP1R) cell line to evaluate human serum tolerance levels. The results show similar EC_{50}'s for all samples tested, ranging from only 514 nM for the control sample (no serum) to 679 nM for the sample containing 33.3% human serum. This indicates HitHunter cAMP assays will tolerate high serum levels while maintaining robust assay performance.

Obtain Excellent Reproducibility and High Sensitivity for Testing Biologics

Perform quality control assays, including potency assays for lot release and stability testing in biologics.



Evaluation of lot-to-lot consistency using a GLP agonist, GLP1 (7-36), and the cAMP Hunter $G\alpha_s$-coupled GLP1R bioassay that incorporates the HitHunter cAMP Assay for Biologics. Results indicate the assay's excellent reproducibility for all three lots tested with S:B ratios over 10 fold and EC_{50}'s ranging from only 760 nM to 860 nM.

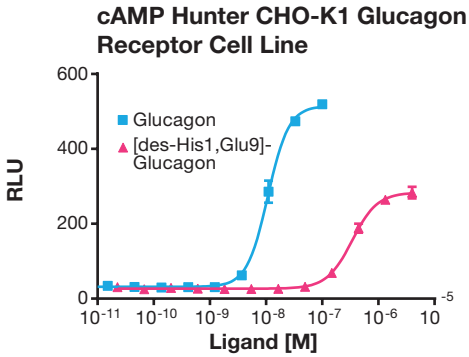


Evaluation of lot-to-lot consistency using a biologic ligand, SDF1α, and the cAMP Hunter $G\alpha_i$-coupled CXCR4 receptor (chemokine C-X-C motif receptor 4) cell line. Results show high sensitivity detection and excellent reproducibility with overlapping S:B ratios of ~6 and EC_{50}'s ranging from only 689 pM to 796 pM.

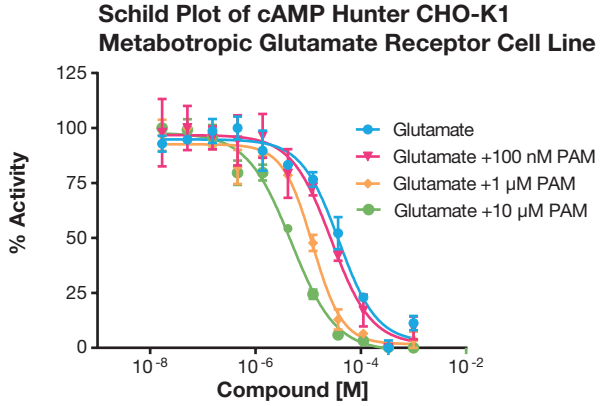
Validated for Accurate Ligand Pharmacology

Identify Allosteric Modulators, Partial Agonists, Inverse Agonists, and Silent Antagonists

HitHunter® cAMP assays' superior assay performance, with large assay windows and wide dynamic range, allows for easy identification of a diverse set of pharmacological ligands such as positive allosteric modulators (PAMs), negative allosteric modulators (NAMs), partial agonists, and more.



Easily identify partial agonists. This experiment uses a cAMP Hunter™ $G\alpha_s$-coupled glucagon receptor (GCGR) CHO-K1 cell line to analyze two agonists. Results reveal [des-His1, Glu9]-glucagon exhibits partial agonism (S:B of 9.2; EC_{50} of 340 nM) compared to the full native agonist, glucagon (S:B of 14.8; EC_{50} of 11 nM).



Schild analysis experiment using the cAMP Hunter CHO-K1 GRM4 cell line to provide quantification of the activity of a novel PAM specific to the metabotropic glutamate receptor 4. Results demonstrate the expected left-shifting of the dose dependent response of the agonist (glutamate) with increasing concentrations of PAM.

To learn about additional cAMP assay applications, download the validation slide set at discoverx.com/cAMP

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