cAMP Hunter™ eXpress GPCR Assay

For chemiluminescent detection of cAMP for GPCR activity

Simple Solutions for Complex Biology

DiscoveRx

Table of Contents

Please read entire booklet before proceeding with the assay.
For additional information or Technical Support, contact info@discoverx.com or visit www.discoverx.com.

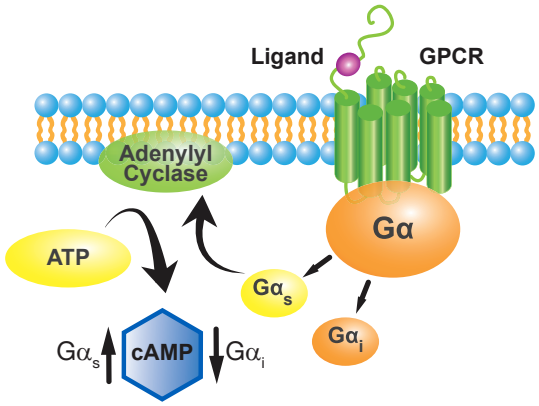
Overview

The cAMP Hunter eXpress GPCR Assay can be used for small or large molecules. The kits provide a robust, highly sensitive and easy-to-use assay for monitoring G-protein coupled receptor (GPCR) activation based on 3'-5'-cyclic adenosine monophosphate (cAMP) production in cells.

Technology Principle

GPCR activation following ligand binding initiates a series of second messenger cascades that result in a cellular response. The signaling involves a membrane bound enzyme called adenylate cyclase. $G\alpha_i$- and $G\alpha_s$-coupled receptors modulate cAMP by either inhibiting or stimulating adenylate cyclase, respectively. With the cAMP Hunter eXpress GPCR Assay, cells over expressing GPCRs utilize the natural coupling status of the GPCR to monitor activation of $G\alpha_i$- and $G\alpha_s$-coupled receptors. Following ligand stimulation, the functional status of the GPCR is monitored by measuring the cellular cAMP levels using a homogeneous (no wash), gain-of-signal competitive immunoassay based on Enzyme Fragment Complementation (EFC) technology.

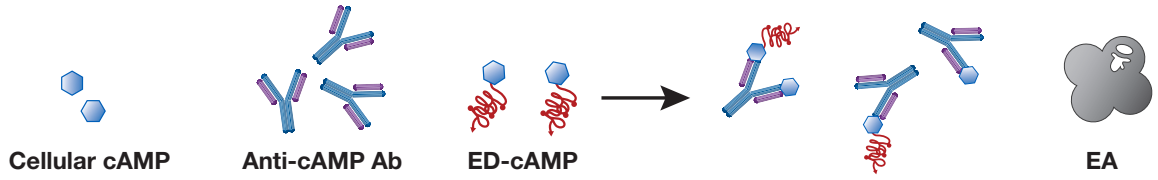
GPCR cAMP Pathway



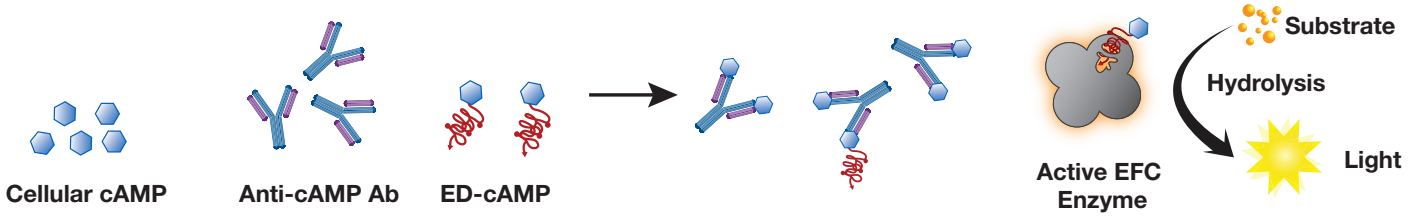
The cAMP Hunter eXpress GPCR Assay EFC technology uses a β-galactosidase (β-gal) enzyme split into two fragments, the Enzyme Donor (ED) and Enzyme Acceptor (EA). Independently these fragments have no β-gal activity; however, in solution they rapidly complement to form an active β-gal enzyme.

In this assay, cAMP from cells and ED-labeled cAMP (ED-cAMP) compete for anti-cAMP antibody (Ab) binding sites. Antibody-bound ED-cAMP will not be able to complement with EA, but unbound ED-cAMP is free to complement EA to form active enzyme, which subsequently produces a luminescent signal. The amount of signal produced is directly proportional to the amount of cAMP in the cells.

Low Levels of Cellular cAMP



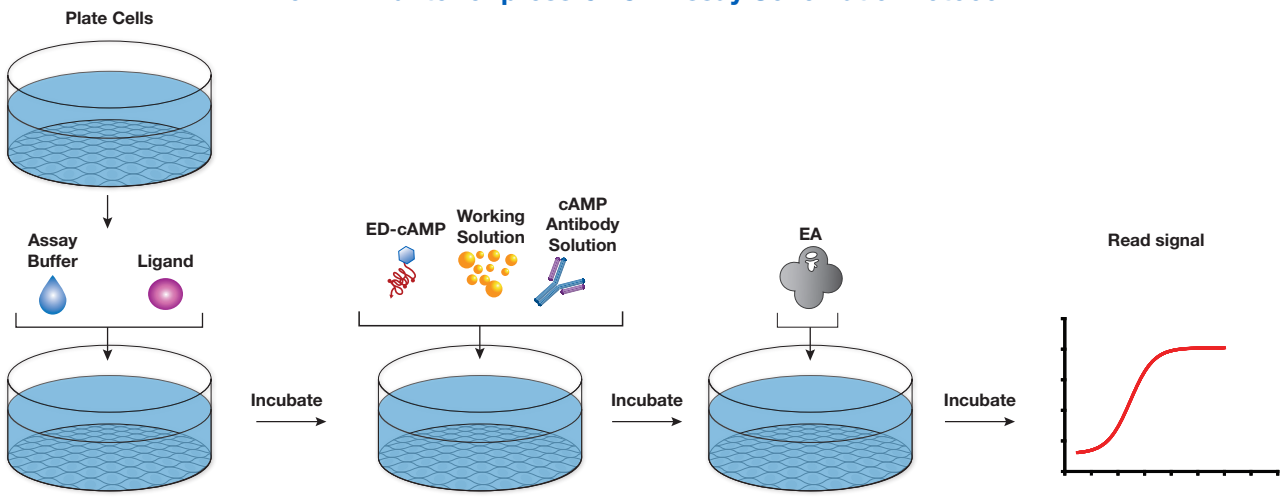
High Levels of Cellular cAMP



Intended Use

The cAMP Hunter eXpress GPCR Assay kits provide a robust, highly sensitive and easy-to-use, cell-based functional assay to study GPCR activity through cAMP production. The pre-validated, frozen eXpress cells have been manufactured for short term use and are provided in a convenient, ready-to-screen format that saves time and adds convenience. The kits contain all the reagents needed for the detection of cAMP from eXpress cells expressing $G\alpha_i$- and $G\alpha_s$-coupled receptors induced with a small or large molecule ligand. This flexible assay system has been designed to work in agonist or antagonist mode for 96- and 384-well plate formats. After plating and stimulation of cells, the user simply adds the assay reagents to the cells following the homogeneous, easy-to-use protocol provided.

cAMP Hunter eXpress GPCR Assay Schematic Protocol



Materials Provided and Storage Conditions

List of Components				
Cell Components	**Number of cells**			**Storage**
cAMP Hunter eXpress Cells	1 vial	2 vials	10 vials	Cells are shipped on dry ice and should arrive in a frozen state. To ensure maximum cell viability, thaw the vials as soon as possible upon receipt. If continued storage of the frozen vials is necessary, store vials at -80°C immediately upon arrival. DO NOT store at –80°C for >2 weeks. For longer term storage (>2 weeks), store vials in the vapor phase of liquid nitrogen (N_2). DO NOT store vials in liquid phase of N_2. (see Safety Warning below)
Number of Cells	3.75 x 10^6 cells each vial in 200 µL	3.75 x 10^6 cells each vial in 200 µL	3.75 x 10^6 cells each vial in 200 µL	
Kit Components	**Volume in each bottle (mL)**			**Storage**
cAMP Lysis Buffer	3.8	7.6	38	Upon arrival, store reagents at ≤-20°C. The detection kit is stable until the expiry date indicated on the kit box outer label. Thaw reagents at room temperature before use. After thawing, store reagents for up to 1 month at 2-8°C. For longer term storage, aliquots of all the components may be re-frozen in opaque containers at ≤-20°C. The reagents can be thawed and frozen for a total of 3 times without loss in performance.
cAMP Solution D	5	10	50	
cAMP Solution A	8	16	80	
cAMP Antibody Reagent	2.5	5	25	
cAMP Standard (250 µM)	1	1	1	
Substrate Reagent 1	1	2	10	
Substrate Reagent 2	0.2	0.4	2	
Forskolin (dried powder)	1 x 0.25 mg	2 x 0.25 mg	10 x 0.25 mg	If Forskolin is not used immediately, prepare multiple aliquots and store at -20°C. Avoid multiple freeze-thaw cycles. When stored properly, the solution is stable for up to 2 months.
Cell Assay Buffer	12.5	25	250	-20°C
Assay Complete™ Cell Plating Reagent	1 x 20 mL	2 x 20 mL	2 x 100 mL	-20°C
Plate Format	**1 plate**	**2 plates**	**10 plates**	Room Temperature
96-well, No. of data points	100	200	1,000	Included: 96-well white-walled clear-bottom tissue culture treated plate
384-well, No. of data points	400	800	4,000	Not Included

Bulk cells are available upon request

Safety Warning: A face shield, gloves and lab coat should be worn at all times when handling frozen vials. The manufacturer of the cryovial recommends storing the vials in the vapor phase above the liquid N_2. Upon thawing, if liquid N_2 is present in the cryovial, it rapidly converts back to its gas phase which can result in the explosion of the vial upon its removal from the freezer

Additional Materials

Required Materials	Ordering Information
Disposable Reagent Reservoir	Thermo Scientific, Cat. No. 8094 or similar
Green V-bottom Ligand Dilution Plates (10 plates/pack)	DiscoveRx Cat. No. 92-0011
15 mL polypropylene tubes and 1.5 mL microtubes	
Tissue culture disposables (pipettes 1 mL – 25 mL)	
Humidified tissue culture incubator (37°C, 5% CO_2)	
Single and multichannel micro-pipettors and pipette tips (10 µL – 100 µL)	
Multimode or luminescence plate reader	

Recommended Materials, Reagents and Equipment	Ordering Information
Control ligands	www.discoverx.com/controlligands
Test Compounds	
IBMX, for $G\alpha_s$ assays	DiscoveRx, Cat. No. 92-0007

Instrument Compatibility Chart	
Compatible with any Luminometer. Select examples indicated below.	
Berthold Technologies: Mithras LB940, CentroLIApc	**Molecular Devices:** FLIPR, SpectraMax M3/ M4/M5/M5e, FlexStation 3, SpectraMax L
Biotek: Synergy 2	**Perkin Elmer:** TopCount, Victor 2 or V, Fusion, LumiCount, Envision, Micro-beta (Trilux), Viewlux, Northstar, EnSpire
BMG: PheraStar, Cytostar, LumiStar	**Promega:** GloMax systems
Caliper: LabChip 3000 & EZ Reader	**Tecan:** Ultra, Evolution
GE: LEAD seeker, Farcyte	**Thermo Scientific:** Luminoskan Ascent
Hamamatzu: FDS6000, FDSS/RayCatcher	**Turner BioSystems:** Modulus Microplate

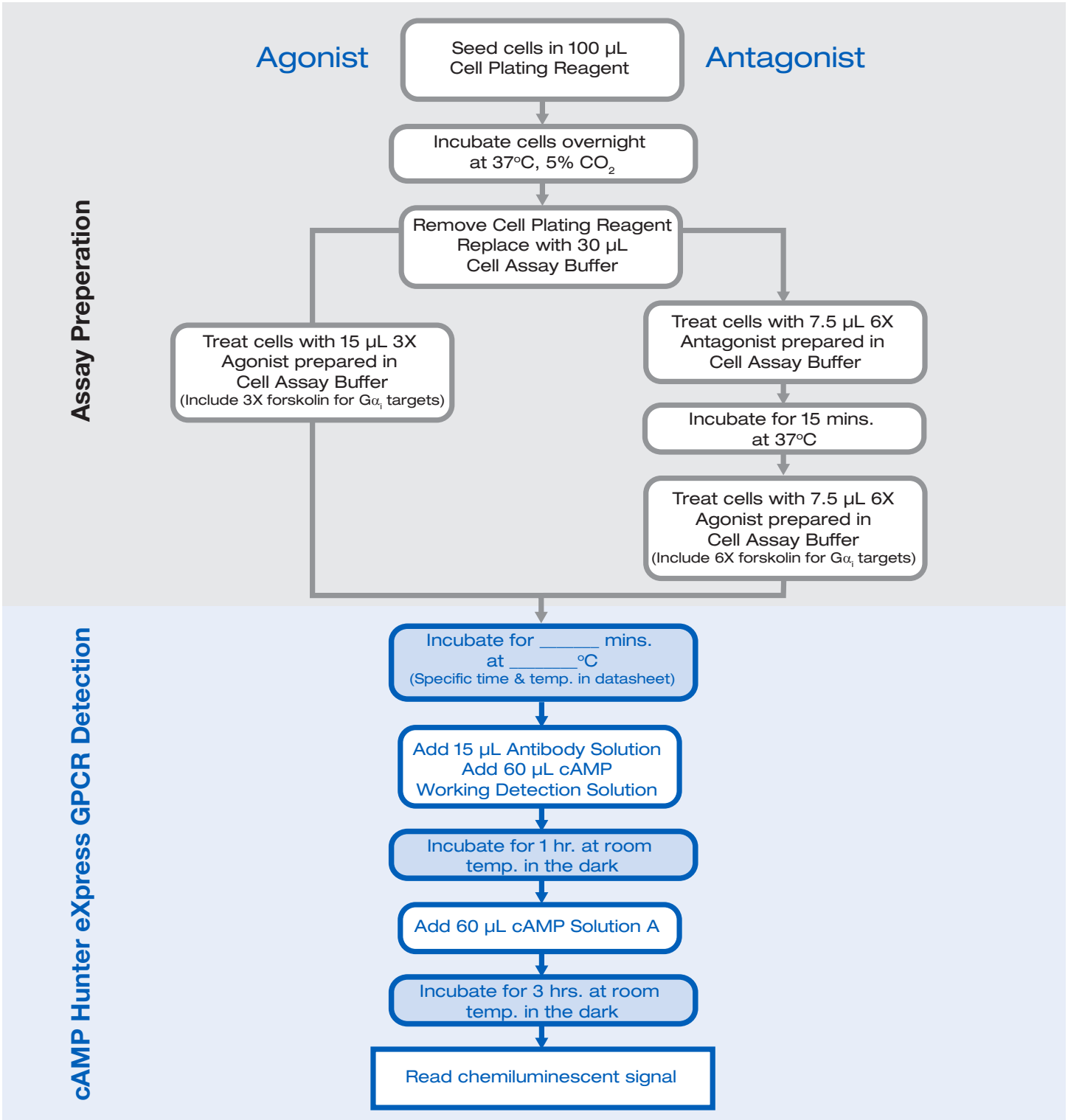
Protocol Schematic

Tip: *Use this sheet to note your assay specific conditions. Post on your bench to use as a quick reference guide.*

Assay Name:_____ Date: _____

Product Details: _____

Quick-Start Procedure: In a white-walled 96-well tissue culture treated plate perform the following:



Detailed Protocol (Agonist, 96-well, $G\alpha_s$)

The following detailed protocol is specific to detecting cAMP in cells stimulated by an agonist for $G\alpha_s$-coupled receptors in a 96-well format plate.

Refer to the appendix for variations (e.g. agonist/$G\alpha_i$-coupled, antagonist, HTS, PAMs, NAMs, crude biologics, and 384-well).

Step 1: Cell Preparation

The following steps outline the procedure for thawing and plating frozen cAMP Hunter eXpress cells from cryogenic vials. Each vial contains sufficient cell numbers to generate (1) 96-well microplate prepared at the seeding density described.

Do not substitute cell plating from an alternate kit at any time

a. Remove the cAMP Hunter eXpress Assay cells cryovials from -80°C or liquid N_2 vapor storage and place them immediately on dry ice prior to thawing.

Do not expose vials to room temperature

Safety Warning: A face shield, gloves and lab coat should be worn at all times when handling frozen vials. The manufacturer of the cryovial recommends storing the vials in the vapor phase above the liquid N_2. Upon thawing, if liquid N_2 is present in the cryovial, it rapidly converts back to its gas phase which can result in the explosion of the vial upon its removal from the freezer

b. Decontaminate the vial by spraying and wiping with 70% ethanol. All of the procedures from this point on should be carried out under aseptic conditions in a culture hood.

c. Pre-warm Assay Complete™ Cell Plating Reagent in a 37˚C water bath for 15 mins.

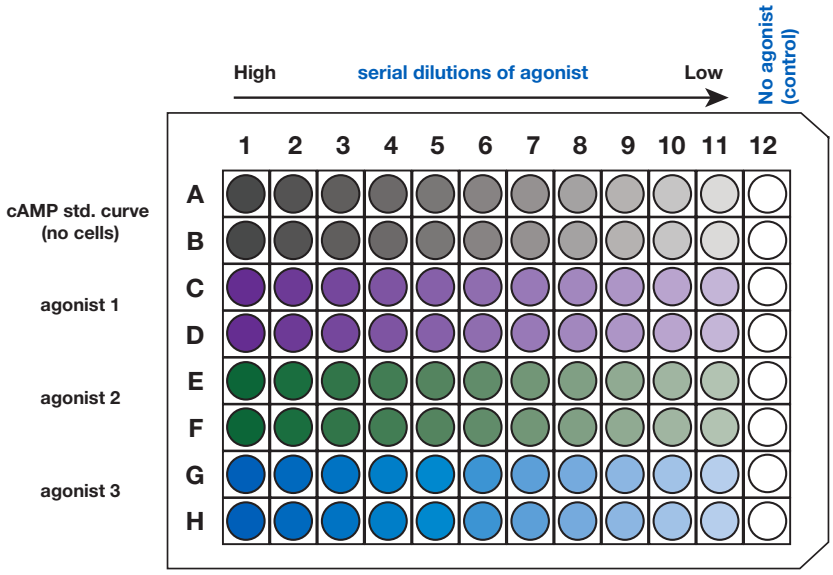
d. Add 0.5 mL of pre-warmed AssayComplete Cell Plating Reagent to the cell vial to thaw the cells. Pipette up and down gently several times to ensure that cells are evenly distributed.

Do not thaw vials in 37°C water bath or centrifuge

e. Immediately transfer the cells to 11.5 mL of pre-warmed AssayComplete Cell Plating Reagent. Mix and pour into a disposable reagent reservoir.

f. Prepare the 96-well tissue culture treated assay plate using the plate map figure on the next page. Leave the first 2 rows empty (with no cells) to allow for an 11-point cAMP standard curve in duplicate. For the remaining wells, plate 100 ul of cells into each well. For rows C through H, plate 3 different 11 point agonist curves in duplicate. Optionally, samples can be run on different plates, in triplicates or other variations.

g. Place the seeded plate in a 37°C, 5% CO_2 humidified incubator for 18-24 hours prior to testing (refer to the target-specific datasheet for the recommended incubation time).



Agonist assay plate map: Create 11-point curves for cAMP standard curve and 3 different agonists in duplicate.

Step 2: Assay Plate Preparation

a. Completely remove the cell media from assay plate wells by aspiration

b. Immediately add 30 µL of Cell Assay Buffer to all empty wells in the assay plate.

Removing the media completely is crucial for reducing variability of replicates.

Step 3: cAMP Standard Preparation

When optimizing the assay conditions, always include a cAMP standard curve. The standard curve not only verifies that the kit components are working properly, but also serves as a detection limit guide. If the amount of cAMP being detected exceeds the detection limit of the cAMP detection kit, the EC_{50} will start to shift (depending on the coupling status of $G\alpha_s$ or $G\alpha_i$, the shift will be towards the right or left). To avoid this situation, the cell number per well should be optimized. cAMP standard should be prepared fresh before agonist compound addition.

a. Prepare cAMP standard serial dilutions in a separate plate by diluting the cAMP standard (2.5×10^{-4}M) in a 1:9 ratio (so 1 part cAMP standard plus 8 parts Cell Assay Buffer). This dilution ratio corresponds to the highest standard concentration (well No. 1) at 2.31×10^{-6}M in an assay volume of 180 µL. Make 10 additional 3-fold serial dilutions, using the Cell Assay Buffer, with the last well as the negative control as shown below.

   1. Using a separate dilution plate (or polypropylene tubes), label the wells No. 1 to No. 12.

   2. Add 160 µL of Cell Assay Buffer and 20 µL of cAMP standard to well No. 1 (this will be the highest concentration of cAMP standard).

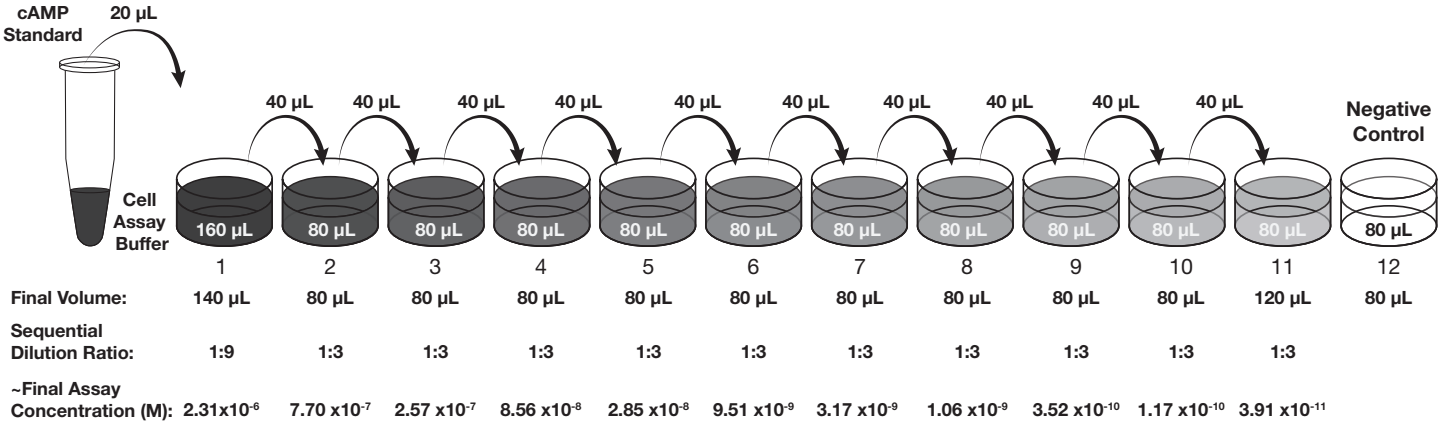
   3. Add 80 µL of Cell Assay Buffer to dilution wells No. 2 to No. 12. This is enough volume for more than 4 rows.

   4. Remove 40 µL from well No. 1 and add it to well No. 2. Mix gently.

   5. With clean tip, remove 40 µL from well No. 2 and add it to well No. 3. Mix gently.

6. Repeat this process until well No. 11. *Do not* add cAMP standard to well No. 12 since this is the negative control well (containing only Cell Assay Buffer).

b. Add 15 µL of cAMP standard dilutions in duplicate to the first two rows (rows A and B) of the assay plate as shown in the previously described assay plate map.



cAMP Standard serial dilutions: Make 11 three fold serial dilutions of cAMP standard solution in a separate plate.

Step 4: Agonist Preparation

a. Prepare agonist serial dilutions in a separate dilution plate in a 11-point series of 3X (3-fold) dilutions of agonist in Cell Assay Buffer or appropriate agonist buffer (as specified on the datasheet). The final concentration of each dilution should be prepared at 3X of the final screening concentration.

For $G\alpha_i$ specific targets or other ligand variations (e.g. antagonists), refer to the appendix section.

1. For each agonist, label the wells of a separate dilution plate (or polypropylene tubes) No. 1 to No. 12.
2. Similar to the cAMP standard serial dilutions, add 80 µL of Cell Assay Buffer (or agonist specific buffer) to dilution wells No. 2 to No. 12. This is enough volume for over 4 rows, and the dilution volume may be adjusted according to the number of wells desired.
3. Prepare the highest concentration of Agonist in Cell Assay Buffer (or agonist specific buffer). We recommend preparing a final screening concentration that is 250X the expected EC_{50} of the agonist. Therefore, prepare a working concentration that is 750X the expected EC_{50} per well. E.g. for an expected EC_{50} of 1 nM, prepare the highest working concentration at 750 nM. This is 3X the screening or final top concentration of 250 nM, and the expected EC_{50} concentration will lie near the center of the dose response curve.
4. Add 120 µL of the highest concentration of Agonist/Cell Assay Buffer to well No. 1.
5. Remove 40 µL from well No. 1 and add it to well No. 2. Mix gently.
6. With clean tip, remove 40 µL from well No. 2 and add it to well No. 3. Mix gently.
7. Repeat process until well No. 11. *Do not* add agonist to well No. 12 since this is the negative control well.
8. Set up additional serial dilutions for additional agonists in a similar manner.

b. Add 15 µL of each 3X agonist serial dilution in duplicate to the designated agonist rows (e.g. agonist 1 in rows C and D; agonist 2 in rows E and F; and agonist 3 in rows G and H) of the assay plate as indicated on the previously described assay plate map.

c. Incubate assay plate at the indicated time and temperature for the specific cell line (please refer to the specific cell line datasheet for conditions). For most cell lines, incubate for 30 minutes at 37°C. For the best results, the optional incubation time should be empirically determined.

Step 5: Antibody and Working Solution Addition

a. Following agonist incubation, add 15 µL of cAMP Antibody Reagent to all wells.

b. Prepare a stock of cAMP Working Detection Solution in a separate 15 ml polypropylene tube, by mixing 19 parts of cAMP Lysis Buffer, 5 parts of Substrate Reagent 1, 1 part Substrate Reagent 2, and 25 parts of cAMP Solution D.

c. Add 60 µL of cAMP Working Detection Solution to all wells of the assay plate. *Do not* pipette up and down in the vial to mix or vortex plates.

d. Incubate assay plate for 1 hour at room temperature in the dark for the immunocompetition reaction to occur.

Make stock within 8 hours of use.

cAMP Working Detection Solution		
Components	**Volume ratio**	**Volume per plate**
cAMP Lysis Buffer	19	3.8 ml
Substrate Reagent 1	5	1.0 mL
Substrate Reagent 2	1	0.2 mL
cAMP Solution D	25	5.0 mL
Total Volume		**10 mL**

cAMP Working Detection Solution is light sensitive, thus incubation in the dark is necessary.

Step 6: Enzyme Acceptor Addition

a. Add 60 µL of cAMP Solution A to all wells of the assay plate. *Do not* pipette up and down in the vial to mix or vortex plates

b. Incubate assay plate for 3 hours at room temperature in the dark.

cAMP Working Detection Solution is light sensitive, thus incubation in the dark is necessary.

Step 7: Assay Plate Reading

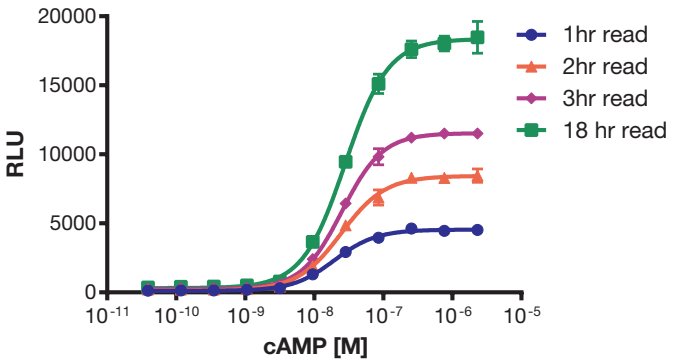
a. Read samples on a standard luminescence plate reader at 0.1 to 1 second/well for photomultiplier tube readers or 5-10 seconds for imager. The plate may be incubated overnight and the signal may be measured the next day. In general, the signal continues to increase and reaches a maximum approximately 3-6 hours after the last incubation step. The actual signal characteristics over time are affected by lab conditions, such as temperature, and the user should establish an optimal read time. Luminescence readout usually collects signal from all wavelengths. Some instrument manufacturer may include a cutoff filter at high wavelengths, but usually no wavelength setting is needed for luminescence readout.

b. Data analysis can be performed using your choice of statistical analysis software (e.g. GraphPad Prism, Molecular Devices Softmax Pro, Biotek Instruments Gen5, Microsoft Excel, etc.).

Typical Results

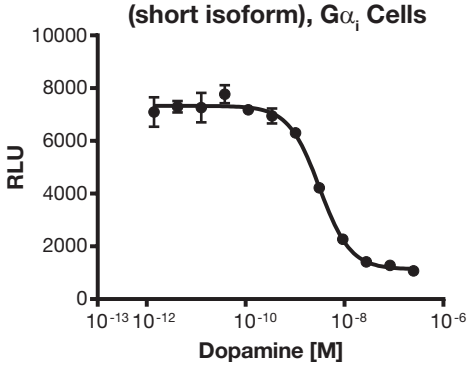
Typical results shown below of the cAMP standard curve analysis (top left) and cAMP Hunter eXpress GPCR Assay using the cAMP Hunter™ dopamine receptor D2 (short isoform) $G\alpha_i$ cells (top right) and glucagon-like peptide receptor 1 $G\alpha_s$ cells (bottom). Note for the cAMP standard curve graph, the signal continues to increase over time and plates can be read the following day.

cAMP Standard Curve (96 well)



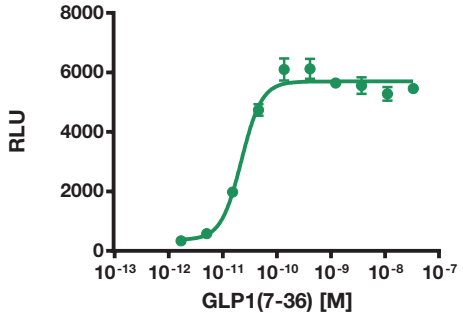
	1 hr. read	2 hr. read	3 hr. read	18 hr. read
EC_{50}	$1.96x10^{-8}$	$2.47x10^{-8}$	$2.53x10^{-8}$	$1.82x10^{-8}$
S/B	37.6	42.2	43.1	50.4

Dopamine Receptor D2 (short isoform), $G\alpha_i$ Cells



EC_{50}	$1.40x10^{-9}$
S/B	7.1

Glucagon-like Peptide Receptor 1, $G\alpha_s$ Cells



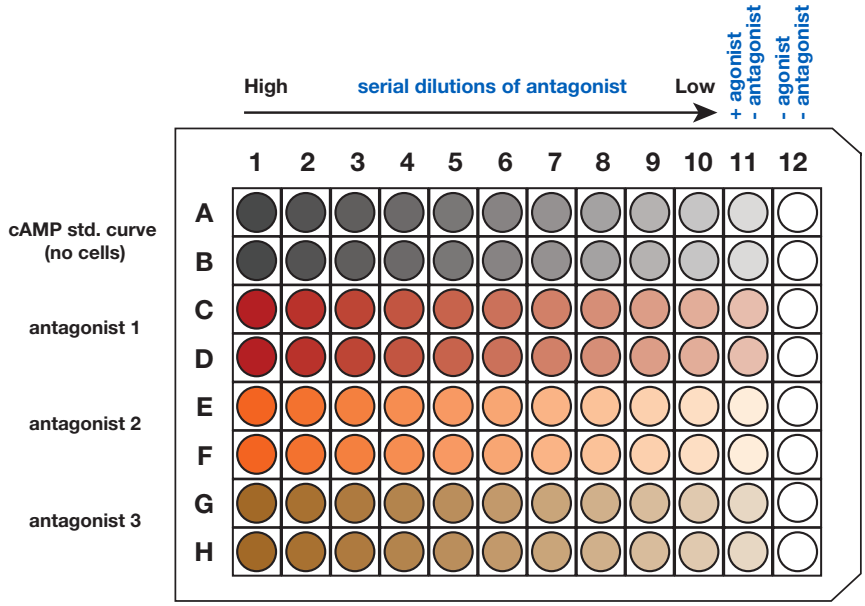
EC_{50}	$2.21x10^{-11}$
S/B	18.0

Appendix

Antagonist Protocol for $G\alpha_s$ Receptors

Protocol Modification

1. Antagonist serial dilution: Similar to the agonist step 4a, prepare a 10-point series of 3-fold dilutions of antagonist in Cell Assay Buffer. The concentration of each dilution should be prepared at 6X of the final screening concentration. See plate map below for an antagonist set-up example.



Antagonist assay plate map: Create 10-point curves with cAMP standard curve and 3 different antagonists in duplicate.

2. Antagonist/Agonist addition: Replace step 4b of the agonist detailed protocol with the following directions.

   a. Add 7.5 µl of each 6X antagonist serial dilution to the designated antagonist rows of the assay plate.

   b. Incubate assay plate for 15 minutes at 37°C. For best results, the optimal incubation time should be empirically determined.

   c. For the agonist challenge, add 7.5 µL of agonist at 6X the EC_{80}. We recommend running the agonist dose response curve to determine the EC_{80}. The specific concentration of agonist to be used can vary from EC_{75} to EC_{100}, based on the target and assay conditions. For $G\alpha_i$ targets, instead of the agonist alone, add 7.5 µL of 6X forskolin plus agonist solution at this time.

   For $G\alpha_i$ targets, only add the forskolin during the agonist challenge step.

   d. Continue with step 4c (incubation) of the agonist detailed protocol.

Protocol for $G\alpha_i$-Coupled Receptors

In order to measure $G\alpha_i$-coupled receptors (targets), the agonist sample is added in the presence of forskolin. Forskolin activates adenylate cyclase and increases intracellular levels of cAMP. For a $G\alpha_i$ receptor, agonist binding or activation inhibits the intracellular cAMP accumulation induced by forskolin. As a result, the dose response curve generated in the presence of agonist plus forskolin will have a negative slope. Since forskolin treatment results in increased intracellular cAMP levels in all cAMP Hunter™ cells, it can serve as a positive control for the performance of the cells and detection reagents

Protocol Modification

1. Replace step 4a of the agonist detailed protocol with the following steps to prepare a $G\alpha_i$–coupled receptor ligand plus forskolin 11-point serial dilution.

   For antagonist $G\alpha_i$-coupled receptors, forskolin is added to the agonist challenge solution.

   a. Prepare a 3X (for agonist mode) or 6X (for antagonist mode) final screening concentration of forskolin. Refer to the target-specific datasheet for recommended concentrations of forskolin. A general recommendation for the final forskolin concentration is 10-15 µM for CHO-K1 cells and 15-20 µM for HEK 293. For example, if the forskolin final in-well screening concentration is recommended to start at 15 µM, prepare a 45 µM forskolin solution (which is 3X).

   For forskolin, avoid multiple freeze/thaw cycles. Store as multiple aliquots at -20°C for up to 2 months.

   b. Prepare the highest concentration, 3X (for agonist mode) or 6X (for antagonist mode), of $G\alpha_i$ agonist in the forskolin solution prepared above.

   c. Add 120 µL of the highest concentration of agonist plus forskolin to well No. 1.

   d. Remove 40 µL from well No. 1 and add it to well No. 2. Mix gently.

   e. With clean tip, remove 40 µL from well No. 2 and add it to well No. 3. Mix gently.

   f. Repeat process until well No. 11. Do not add agonist to well No. 12 since this is the negative control well.

   g. Set up additional serial dilutions for additional agonists in a similar manner.

2. Replace step 4b of the agonist detailed protocol. Add 15 µL (for agonist mode) or 7.5 µL (for antagonist mode) of the $G\alpha_i$ agonist plus forskolin serial dilutions in duplicate to the designated agonist rows (e.g. agonist 1 in rows C and D; agonist 2 in rows E and F; and agonist 3 in rows G and H) of the assay plate as indicated on the previously described assay plate map.

3. Continue with step 4c (incubation) of the agonist detailed protocol.

PAMs and NAMs

For positive allosteric modulators (PAMs), refer to the antagonist protocol above, but change the agonist challenge concentration to EC_{20} instead of EC_{80}.

For negative allosteric modulators (NAMs), refer to the antagonist protocol above with no changes.

High Throughput Screening

For high throughput screening applications, the cAMP Antibody Reagent can be added before ligand introduction to save an addition step. Briefly, pre-dilute the cAMP Antibody Reagent from the kit in a 1:2 ratio with Cell Assay Buffer (so 1 part cAMP Antibody Reagent to 2 parts Cell Assay Buffer). Replace step 2a of the agonist detailed protocol by adding 45 µl of diluted antibody mixture to each well. Replace step 4b by adding 15 µl of 4X agonist to each well. Incubate the assay plate as indicated in step 4c and skip to step 5b of the standard protocol.

Crude Biologic Samples

The cAMP Hunter eXpress GPCR kits can be run in the presence of high levels of serum or plasma without significantly impacting assay performance. Therefore, standard curves and samples can be prepared in neat serum or plasma and added directly to cells without further dilution. For the best results, the optimized minimum required dilution (MRD) of crude samples should be empirically determined.

Adjusted Volumes for 384-well Format

Use the following table to adjust the component volumes per well for 384-well plates.

384-well Format		
Assay Reagents	**96 plate (per well)**	**384 plate (per well)**
No. of Cells	**30,000 ***	**10,000 ***
AssayComplete Cell Plating Reagent	100 µL **	20 µL **
Cell Assay Buffer	30 µL	10 µL
Ligand (e.g. Agonist)	15 µL	5 µL
cAMP Antibody Reagent	15 µL	5 µL
cAMP Working Detection Solution	60 µL	20 µL
cAMP Solution A	60 µL	20 µL
Final Assay Volume **	**180 µL**	**60 µL**

* The number of cells is dependent on the cell line.

** The AssayComplete Cell Plating Reagent volume is used to plate cells and this volume is removed during first step.

FAQs

Is IBMX necessary in the DiscoveRx cAMP kits?

- IBMX is not necessary and we do not use it for our internal testing; however, the kit is compatible with the use of IBMX. If IBMX is to be used, cell number per well and IBMX concentration needs to be optimized so that the amount of cAMP generated stays within the optimal detection of the assay kit.

How do I use suspension cells?

- Harvest and resuspend suspension cells in Cell Assay Buffer media (either 1X HBSS + 10mM HEPES or PBS) at the optimal cell density. Typical suspension cell density is approximately 20,000 cells per well in a standard 96-well plate with a cell viability > 90%.

What if there is no or low signal?

- If plated on clear-bottom assay plates, visually inspect the cells before and after running the assay to ensure that they are healthy. Make sure proper cell plating procedure is followed to ensure assay performance.
- Make sure detection reagents are stored and prepared properly.
- Make sure forskolin is included for $G\alpha_i$ coupled receptors. Please refer to the Appendix for $G\alpha_i$ coupled receptor assay protocol.
- Make sure the proper assay mode is used (agonist mode or antagonist mode).
- Make sure the plate reader is setup properly to read luminescence.
- High levels of protein in the wells may interfere with enzyme fragment complementation. If high levels are present, a media exchange step could be performed just prior to the detection reagent addition.

What if the response is lower than expected (lower than expected S:B)?

- Make sure that the ligand is prepared properly, take extra care to observe ligand solubility.
- Make sure DMSO and other solvents are not too high and not more than 1% final concentration.
- When testing ligands at high concentrations, make sure the vehicle is compatible.
- Make sure ligand is incubated for the designated time and at designated temperature.
- Make sure plates are protected from light during incubation.

What if the EC_{50} does not match reported values?

- Include a cAMP standard curve in the assay to ensure that the ligand dose curve is in the dynamic range of the cAMP detection kit. Moving out of the dynamic range may shift the EC_{50} of ligands.
- Make sure ligands are incubated at the proper temperature.
- The source of a ligand, particularly for biologics, can have a large effect on the observed pharmacology.
- Changing tips during serial dilutions can help to avoid carryover.
- Receptor expression level may cause receptor reserve issues in ligand testing. Select a cell line that has medium to low expression of receptors.

What if the variability between replicates is high?

- Make sure dispensing equipment is properly calibrated, and proper pipetting technique is used.

What if my compound is in media containing high concentrations of serum, can I use it as is or will the serum interfere with the assay?

- Our assays are highly tolerant to high serum content (as high as 80% serum). We recommend that you aspirate the high serum media prior to adding detection reagents.

For additional information or Technical Support, contact info@discoverx.com or visit www.discoverx.com.

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