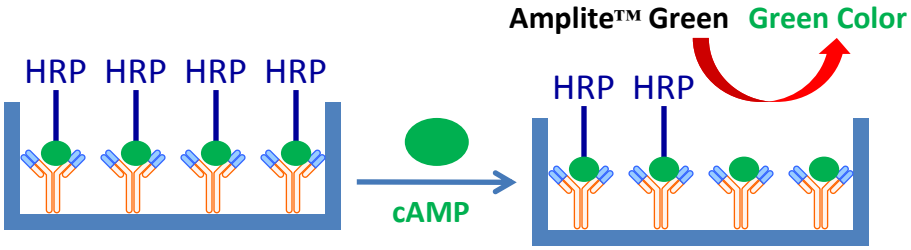
Screen Quest™ Colorimetric ELISA cAMP Assay Kit
Green Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36370 (1 plate), 36371 (10 plates)	Keep in 4°C Avoid exposure to light	Absorbance microplate reader

Introduction

Adenosine 3', 5' cyclic monophosphate (cAMP) is an important second messenger in intracellular signal transduction. Monitoring cAMP levels is one of the most common ways to screen for agonists and antagonists of GPCRs. Screen Quest™ Colorimetric ELISA cAMP Assay Kit is based on the competition between HRP-labeled cAMP and free cAMP for a fixed number of cAMP antibody binding sites. HRP-cAMP is displaced from the HRP-cAMP/anti-cAMP antibody complex by unlabeled free cAMP. In the absence of cAMP, HRP-cAMP conjugate is bound to anti-cAMP antibody exclusively. However, the unlabeled free cAMP in the test samples competes for anti-cAMP antibody with the HRP-cAMP antibody conjugate, therefore inhibits the binding of HRP-cAMP to anti-cAMP antibody.



Maxium HRP activity (No cAMP) Decreasing HRP activity (Increasing free cAMP)

Our Screen Quest™ Colorimetric ELISA cAMP Assay Kit provides the sensitive method for detecting adenylate cyclase activity in biochemical or cell-based assay system. Compared to other ELISA cAMP assay kits, our kit eliminates the tedious acetylation step, and provides the ready-to-use Anti-cAMP Ab coated 96-well plate and HRP substrate Amplite™ Green to quantify the HRP activity. The color product formed is proportional to the activity of HRP-cAMP.

Kit Components

Components	Amount	
	Cat. # 36370 (1 plate)	**Cat. # 36371 (10 plates)**
Component A: cAMP Standard	1 vial (33 µg)	1 vial (33 µg)
Component B: Assay Buffer	20 mL	2x100 mL
Component C: HRP-cAMP Conjugate	1 vial	1 vial
Component D: 10X Wash Solution	10 mL	100 mL
Component E: Cell Lysis Buffer	10 mL	100 mL
Component F: Anti-cAMP Ab Coated 96-Well Plate	1 plate	10 plates
Component G: Amplite™ Green	10 mL	100 mL

Note: Do not freeze Anti-cAMP Ab Pre-coated 96-well plate (Component F), store it at 4°C.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Assay Protocol for One 96-well Plate

Brief Summary

Prepare samples→ Add 75 µL/well of cAMP standard or test samples into the anti-cAMP coated 96-well plate → Incubate at RT for 5-10 min → Add 25 µL/well of 1X HRP-cAMP conjugate → Incubate at RT for 3 hours → Wash 4 times with 200 µL/well Washing Buffer→ Add 100 µL/well of Amplite™ Green → Incubate at room temperature for 1 to 3 hours → Monitor absorbance increase at 405, 650 or 740 nm

Note 1. Allow all the kit components to warm to room temperature before using them;
Note 2: Some material might be stick to the vial cap during the shipment. Briefly centrifuge the vial to collect all the content.

1. Prepare samples:

1.1 **Cell Samples:**
For adherent cells: Plate cells overnight in growth medium at 30,000 -100,000 cells/well for a 96-well plate.
For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 100,000-300,000 cells/well for a 96-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.
Treat cells as desired: The following is an example of Hela cells treated with Forskolin to induce cAMP in a 96-well plate format.
a). Aspirate off cell growth medium, add 100 µL/well 100 µM Forskolin in Hanks and 20 mM Hepes buffer (HHBS), incubate in a 5% CO_2, 37 ^oC incubator for 15 minutes; b). Aspirate off cell solution after the incubation, add 100 µL/well of Cell Lysis Buffer (Component E), and incubate at room temperature for another 10 minutes. This cell lysate can be assayed directly or after diluted in Assay Buffer (Component B).
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density. Cells may be seeded the day before or on the day of the experiment depending upon the cell type and/or the effect of the test compounds.

1.2 **Tissue Samples:** It is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen) due to quick metabolism of cyclic nucleotides in tissue. Weigh the frozen tissue and add 10-20 µL/mg of cell lysis buffer. Homogenize the sample on ice. Spin at top speed for 5 minutes and collect the supernatant. The supernatant may be assayed directly.

1.3 **Urine, Plasma and Culture Medium Samples:** Urine and plasma may be tested directly with 1:200 to 1:1000 dilutions in 1X Lysis Buffer. Culture medium can also be tested with 1:10 to 1:200 dilutions in Lysis Buffer.
Note: RPMI medium may contain > 350 fmol/µL cAMP.

2. Prepare cAMP assay solutions:

2.1 Prepare 100 µM cAMP stock solution by adding 1 mL of Assay Buffer (Component B) to the vial of cAMP Standard (Component A). Make 1:10, 1:100 and 1:3 serial dilutions in Assay Buffer (Component B) to have 10,000, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003 and 0 nM cAMP diluted solutions. Store on ice or 4^{o}C.
Note: The unused reconstituted 100 µM cAMP stock solution should be aliquoted and stored at -20 ^oC.

2.2 Prepare 50X HRP-cAMP conjugate stock solution by adding 55 µL (for Kit 36370) or 550 µL (for Kit 36371) of Assay Buffer (Component B) into the vial of HRP-cAMP Conjugate (Component C). Make 1:50 dilution with Assay Buffer (Component B) to have 1X HRP-cAMP conjugate working solution before use. Store it on ice or 4^{o}C.
Note 1: 25 µL of 1X HRP-cAMP conjugate working solution is enough for one assay point; prepare appropriately volume for single use only.
Note 2: The unused 50X HRP-cAMP conjugate stock solution should be divided into single use aliquots and stored them at -20 ^oC.

2.3 Prepare 1X washing solution by adding 1 mL of 10X Wash Solution (Component D) to 9 mL distilled water.

3. Run cAMP assay:

3.1 All the assay wells will be prepared in the following orders: A) cAMP standards, control, or tests samples; B) HRP-cAMP conjugate.

3.2 Add 75 µL/well of the cAMP diluted standard solution (from Step 2.1) and test samples into each well of the anti-cAMP Ab coated 96-well plate (Component F). We recommended duplicating the assays for each standard and testing sample. Incubate at room temperature for 5 to 10 minutes.

3.3 Add 25 µL/well of 1X HRP-cAMP conjugate working solution (from Step 2.2). Incubate at room temperature for 3 hours by placing the plate on shaker.

3.4 Aspirate plate contents, and wash 4 times with 200 µL/well of 1X wash solution (from Step 2.3).

3.5 Add 100 µL/well of Amplite™ Green (Component G) into each well, and incubate at room temperature for 60 min to 3 hours, protected from light.

3.6 Monitor the absorbance increase at 405nm, 650 nm, or 740 nm using an absorbance plate reader.

Data Analysis

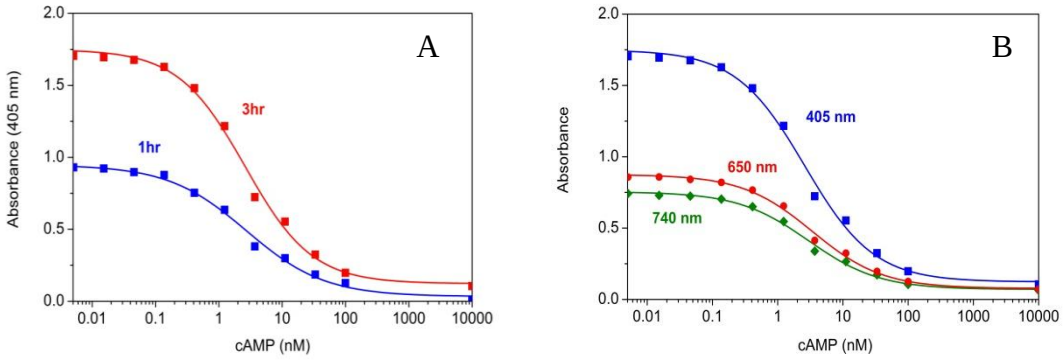


Figure 1. cAMP dose response was measured with Screen Quest™ Colorimetric ELISA cAMP Assay Kit in a clear 96-well plate with a SpectraMax microplate reader. A: The kit can detect as low as 0.1 nM cAMP in a 100 µL reaction volume at 405nm after incubation with Amplite™ Green for 1 hour (blue line) and 3 hours (red line). B: The Absorbance can be read at 405 nm (blue line), 650 nm (red line) or 740 nm (Green line), the data in figure B are from the incubation with Amplite™ Green for 3 hours.

References

1. Alonso GD, Schoijet AC, Torres HN, Flawia MM. (2006) TcPDE4, a novel membrane-associated cAMP-specific phosphodiesterase from Trypanosoma cruzi. Mol Biochem Parasitol, 145, 40.
2. Bader S, Kortholt A, Snippe H, Van Haastert PJ. (2006) DdPDE4, a novel cAMP-specific phosphodiesterase at the surface of dictyostelium cells. J Biol Chem, 281, 20018.
3. Charlie NK, Thomure AM, Schade MA, Miller KG. (2006) The Dunce cAMP phosphodiesterase PDE-4 negatively regulates G alpha(s)-dependent and G alpha(s)-independent cAMP pools in the Caenorhabditis elegans synaptic signaling network. Genetics, 173, 111.
4. Zhang, J. H., Chung, D. Y., Oldenburg, K. R. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screening*, 4: 67-73.