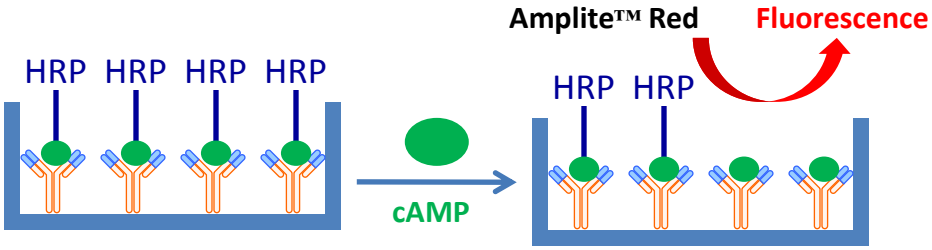
Screen Quest™ Fluorimetric ELISA cAMP Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36373 (1 plate), 36374 (10 plates)	Keep in 4°C Avoid exposure to light	Fluorometric 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™ Fluorimetric 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™ Florimetric 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. The kit uses Amplite™ Red as a fluorimetric substrate to quantify the HRP activity. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation. The fluorescent product formed is proportional to the activity of HRP-cAMP conjugate.

Kit Components

Components	Amount	
	36373 (1 plate)	**36374 (10 plates)**
Component A: cAMP Standard	1 vial (33 µg)	1 vial (33 ug)
Component B: Assay Buffer	20 mL	100 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: 3% H_2O_2	50 µL	250 µL
Component G: Amplite™ Red	1 vial	1 vial
Component H: Anti-cAMP Ab Coated 96-Well Plate	1 plate	10 plates
Component I: Substrate Buffer	10 mL	100 mL

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

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
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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 → Add 25 µL/well of 1X HRP-cAMP conjugate → Incubate at RT for 2 hours → Wash 4 times with 200 µL/well → Add 100 µL/well of Amplite™ Red working solution → Incubate for 15–60 minutes → Monitor fluorescence increase at Ex/Em = 540/590 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 for Hela cells treated with Foskolin 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 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, and 0 nM cAMP diluted solutions. Store on ice or 4^oC.
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 36373) or 550 µL (for Kit 36374) of Assay Buffer (Component B) into the vial of HRP-cAMP Conjugate (Component C). Make 1:50 dilution with assay buffer to have 1X HRP-cAMP conjugate working solution before use. Store it on ice or 4^oC.
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.

2.4 Prepare 200X Amplite™ Red stock solution by adding 50 µL (for Kit 36373) or 500 µL (for Kit 36374) of DMSO into the well of Amplite™ Red (Component G).
Note: 0.5 µL of 200X Amplite™ Red stock solution is enough for one assay point. The unused reconstituted stock solution should be aliquoted and stored at -20 ^oC.

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 solution (from Step 2.1) and test samples into each well of the anti-cAMP Ab coated 96-well plate (Component H). It is recommended to duplicate the assays for each standard and test 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 2 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 Prepare Amplite™ Red working solution by adding 50 µL of 200 X Amplite™ Red stock solution (from Step 2.4) and 11.5 µL of 3% H_2O_2 (Component F) into 10 mL of Substrate Buffer (Component I).
Note: The Amplite™ Red working solution is not stable, use it promptly.
3.6 Add 100 µL/well of Amplite™ Red working solution (from Step 3.5) into each well, and incubate at room temperature for 10 minutes to 2 hours, or up to 24 hours, protected from light.
3.7 Monitor the fluorescence increase at Ex/Em = 540/590 nm (cutoff 570 nm) by using a fluorescence plate reader (top read mode).

Data Analysis

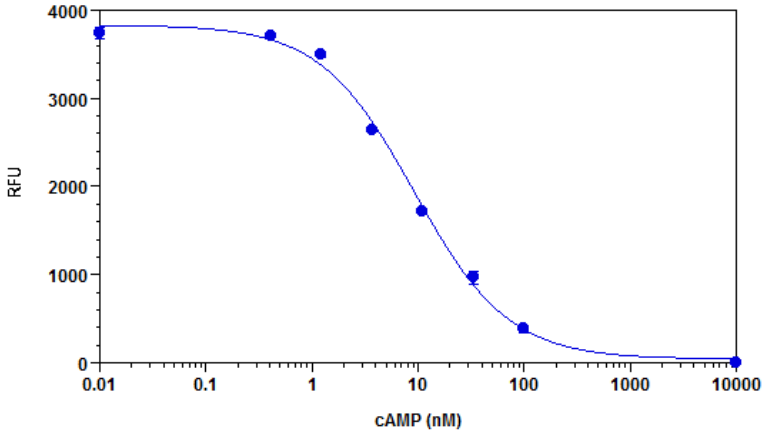

Figure 1. cAMP dose response was measured with Screen Quest™ Fluorimetric ELISA cAMP Assay Kit in a solid black 96-well plate with a Gemini microplate reader. The kit can detect as low as 1 nM cAMP in a 100 µL reaction volume.

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.