Codex™

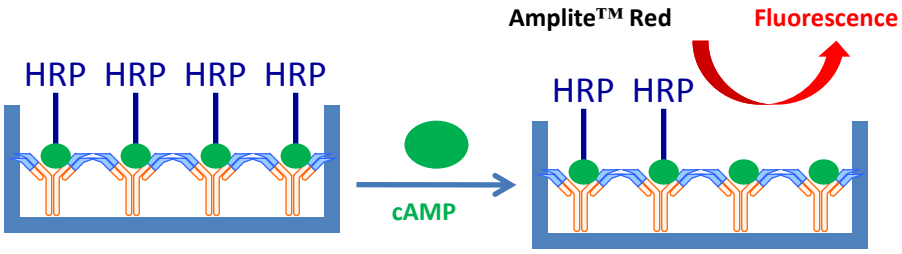
Data Sheet

ACTOne™ cAMP 96-Well Fluorimetric ELISA Assay Kit (10-Plate)

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: CB-80500-513 (10 plates);	Keep in 4°C or -20°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. ACTOne™ cAMP Fluorimetric ELISA Assay Kit is based on the competition between HRP-labeled cAMP and free cAMP for a fixed number of cAMP antibody binding sites. In the absence of free cAMP, HRP-cAMP conjugate is bound to anti-cAMP antibody exclusively. In the presence of free cAMP, HRP-cAMP is displaced from the HRP-cAMP/anti-cAMP antibody complex.



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

Our ACTOne™ cAMP Florimetric ELISA Assay Kit provides the sensitive method for detecting cAMP level 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

Key Components	**CB-80500-513 (Kit for 10 plates)**
Component A: cAMP Standard (100µM)	1 vial 100µl (store at -20°C)
Component B: Assay Buffer	50 mL (store at 4°C)
Component C: 250X HRP-cAMP Conjugate	1 vial 120 µl (store at -20°C)
Component D: 10X Wash Solution	50 mL (store at 4°C)
Component E: Cell Lysis Buffer	110 mL (store at 4°C)
Component F: 3% H_2O_2	1 vial 500 µl (store at 4°C)
Component G: 100X Amplite™ Red	10 vials 100µl (store at -20°C)
Component H: Anti-cAMP Ab Coated 96-Well Plate	10 plates (store at 4°C)
Component I: Substrate Buffer	100 mL (store at 4°C)

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

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Assay Protocol for a 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 80 µL/well → Add 80 µL/well of Amplite™ Red working solution → Incubate for 30–60 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 of 0.75 mM IBMX in Hanks and 20 mM Hepes buffer (HHBS). Incubate at room temperature for 10 minutes; b). Add 50 µL/well of 150 µM Forskolin in HHBS, incubate in a 5% CO_2, 37 ^oC incubator for 15 minutes; c). Aspirate off cell solution after the incubation. Add 100 µL/well of Cell Lysis Buffer (Component E), incubate at room temperature for another 10 minutes. This cell lysate can be assayed directly or diluted in cell lysis buffer.
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 Make serial dilutions of cAMP in Cell Lysis Buffer (Component E) to have 1,000 nM, 300 nM, 100 nM, 30 nM, …….. and 0 nM cAMP diluted solutions. Store on ice or 4^{o}C.
The unused 100 µM cAMP stock solution should be aliquoted and stored at -20 ^{o}C.
2.2 Prepare 1X HRP-cAMP conjugate working solution by adding 10 ul of HRP-cAMP stock solution (250X) in 2.5 ml of 1X Assay Buffer (Component B). Store it on ice or 4^{o}C.
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 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 80 µL/well of 1X wash solution (from Step 2.3).
3.5 Prepare Amplite™ Red working solution by adding 100 µL of 100 X Amplite™ Red stock solution 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 80 µL/well of Amplite™ Red working solution (from Step 3.5) into each well, and incubate at room temperature for 30 minutes to 1 hour.

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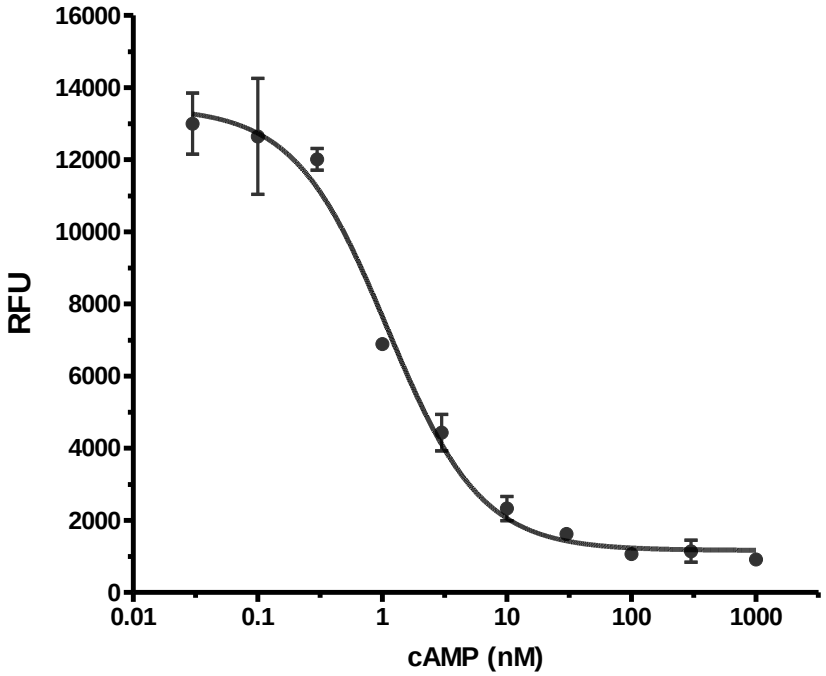
3.7 Monitor the fluorescence increase at Ex/Em = 540/590 nm (cutoff 570 nm) by using a fluorescence plate reader (top read mode).

Data Analysis



cAMP dose response was measured with ACTOne™ cAMP Fluorimetric ELISA Assay Kit in a solid black 96-well plate with a FlexStation Instrument. The kit can detect as low as 0.1 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.

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