

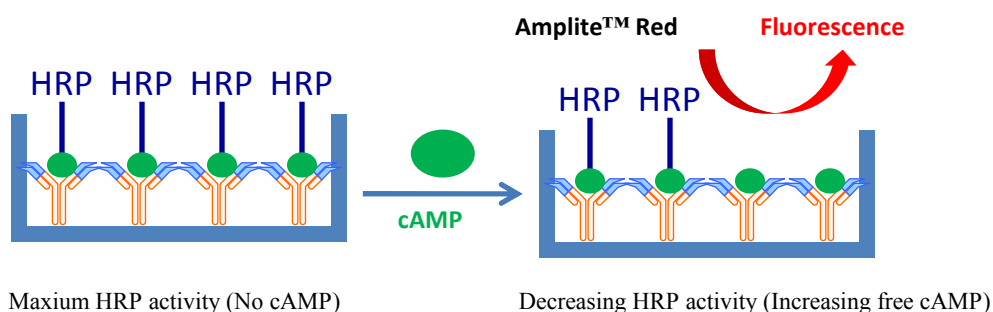
## Data Sheet

## ACTOne™ cAMP Fluorimetric ELISA Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: CB-80500-503 (1 plate); CB-80500-513 (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. 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.



Our ACTOne™ cAMP Fluorimetric 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

Components	Amount	
	CB-80500-503 (1 plate)	CB-80500-513 (10 plates)
Component A: cAMP Standard	1 vial (33 µg)	1 vial (33 ug)
Component B: Assay Buffer	50 mL	500 mL
Component C: HRP-cAMP Conjugate	1 vial	1 vial
Component D: 10X Wash Solution	20 mL	2x100 mL
Component E: Cell Lysis Buffer	10 mL	100 mL
Component F: 3% H <sub>2</sub> O <sub>2</sub>	50 µL	50 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	20 mL	2x100 mL

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

## Assay Protocol for a 96-well Plate

### Brief Summary

Prepare samples → Add 75  $\mu\text{L}$ /well of cAMP standard or test samples into the anti-cAMP coated 96-well plate → Add 25  $\mu\text{L}$ /well of 1X HRP-cAMP conjugate → Incubate at RT for 2 hours → Wash 4 times with 200  $\mu\text{L}$ /well → Add 90  $\mu\text{L}$ /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 stuck 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 Forskolin to induce cAMP in a 96-well plate format.

a). Aspirate off cell growth medium, add 100  $\mu\text{L}$ /well of 0.75 mM IBMX in Hanks and 20 mM Hepes buffer (HHBS). Incubate at room temperature for 10 minutes; b). Add 50  $\mu\text{L}$ /well of 150  $\mu\text{M}$  Forskolin in HHBS, incubate in a 5%  $\text{CO}_2$ , 37  $^\circ\text{C}$  incubator for 15 minutes; c). Aspirate off cell solution after the incubation. Add 100  $\mu\text{L}$ /well of Cell Lysis Buffer (Component F), 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  $\mu\text{L}$ /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/ $\mu\text{L}$  cAMP.*

### 2. Prepare cAMP assay solutions:

2.1 Prepare 100  $\mu\text{M}$  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 $^\circ\text{C}$ .

*Note: The unused reconstituted 100  $\mu\text{M}$  cAMP stock solution should be aliquoted and stored at -20  $^\circ\text{C}$ .*

2.2 Prepare 50X HRP-cAMP conjugate stock solution by adding 55  $\mu\text{L}$  (for CB-80500-503) or 550  $\mu\text{L}$  (for CB-80500-513) 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 $^\circ\text{C}$ .

*Note 1: 25  $\mu\text{L}$  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  $^\circ\text{C}$ .*

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  $\mu\text{L}$  (for CB-80500-503) or 500  $\mu\text{L}$  (for CB-80500-513) of DMSO into the well of Amplite™ Red (Component G).

*Note: 0.5  $\mu\text{L}$  of 200X Amplite™ Red stock solution is enough for one assay point. The unused reconstituted stock solution should be aliquoted and stored at -20  $^\circ\text{C}$ .*

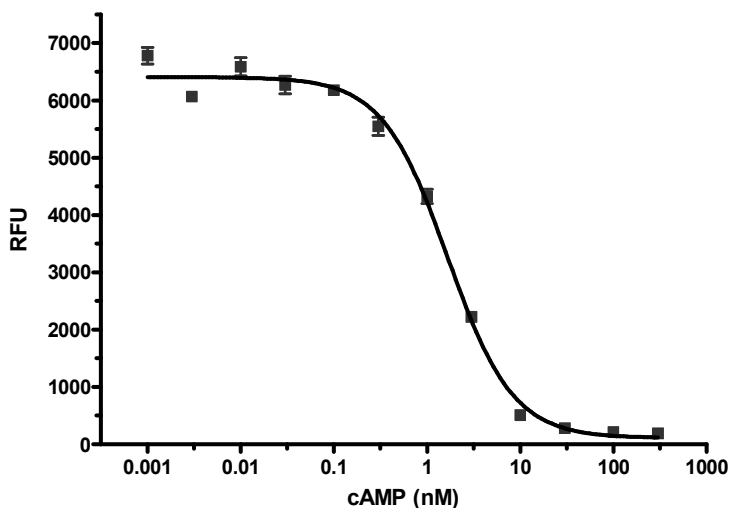
### 3. Run cAMP assay:

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  $\mu\text{L}$ /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  $\mu\text{L}$ /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  $\mu\text{L}$ /well of 1X wash solution (from Step 2.3).
- 3.5 Prepare Amplitude™ Red working solution by adding 50  $\mu\text{L}$  of 200 X Amplitude™ Red stock solution (from Step 2.4) and 11.5  $\mu\text{L}$  of 3%  $\text{H}_2\text{O}_2$  (Component F) into 10 mL of Substrate Buffer (Component I).  
*Note: The Amplitude™ Red working solution is not stable, use it promptly.*
- 3.6 Add 100  $\mu\text{L}$ /well of Amplitude™ 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



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  $\mu\text{L}$  reaction volume.

## References

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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.

**Warning: This kit is only sold to end users. It is covered by a pending patent. Neither resale nor transfer to a third party is allowed without written permission from Codex BioSolutions. Chemical analysis of the kit components is strictly prohibited.**

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