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**Product Manual**

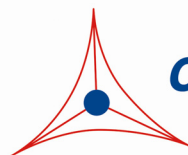
# **cAMP ELISA Kit (Colorimetric)**

## **Catalog Numbers**

<b>STA-500</b>	<b>96 assays</b>
<b>STA-500-5</b>	<b>5 x 96 assays</b>

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **Introduction**

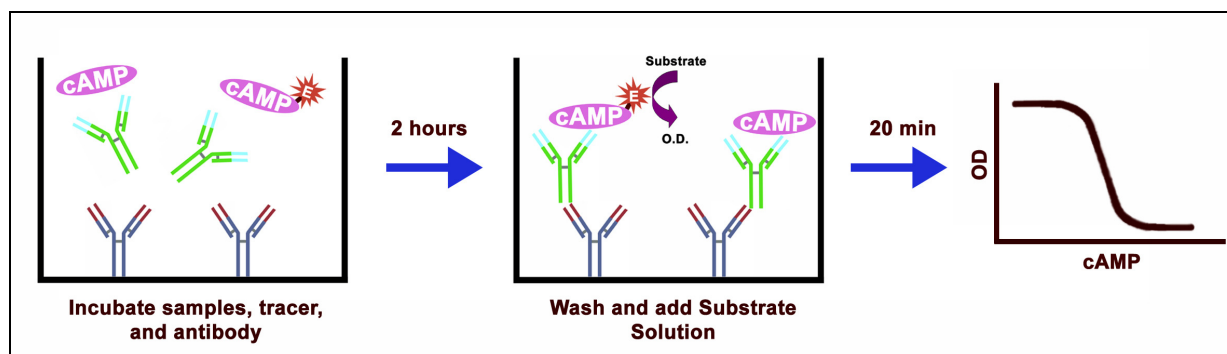
Adenosine 3',5'-cyclic monophosphate (cAMP) is a ubiquitous second messenger involved in various cellular activities in many cell and tissue types. It is converted from adenosine triphosphate (ATP) via adenylyl cyclases (AC), and is inactivated by hydrolysis to 5'-AMP by the actions of phosphodiesterases. cAMP may affect cellular function through several different mechanisms including the activation of cAMP-dependent Protein Kinase (PKA), Guanine Nucleotide Exchange Factors (GEFs), and Cyclic Nucleotide-gated (CNG) channels. PKA is a heterotetramer consisting of 2 regulatory (R) subunits and 2 catalytic (C) subunits. Two cAMP molecules bind cooperatively to 2 sites on each R subunit, releasing the active C subunit monomers to phosphorylate a range of downstream substrates. GEFs facilitate the exchange of GDP for GTP and, therefore, promote the activity of G proteins. Exchange Protein Activated by cAMP (Epac) 1 and 2 are GEFs activated upon binding to cAMP. Epac 1 and 2 have been implicated in regulating the activity of the small GTPase Rap-1 (26, 27). CNG channels are cation channels activated by cGMP and/or cAMP. These channels regulate membrane potential, and due to their  $\text{Ca}^{2+}$  permeability, can alter the levels of intracellular  $\text{Ca}^{2+}$ .

Cell Biolabs' cAMP ELISA Kit is a competitive enzyme immunoassay designed to measure cAMP in cell culture supernatants, plasma, serum, saliva, urine, and cell lysates. The kit selectively measures cAMP levels without any significant cross reactivities to other nucleotides or cyclic nucleotides. The kit has a detection range of 1 pmol/mL to 1000 pmol/mL cAMP.

## **Assay Principle**

An anti-Rabbit IgG polyclonal coating antibody is adsorbed onto a microtiter plate. Cyclic AMP present in the sample or standard competes with Peroxidase cAMP Tracer for plate binding, in the presence of Rabbit Anti-cAMP Polyclonal Antibody.

Following incubation and wash steps, any Peroxidase cAMP Tracer bound to the plate is detected with addition of Substrate Solution. The colored product formed is inversely proportional to the amount of cAMP present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from cAMP Standard and sample concentration is then determined.



## **Related Products**

1. STA-501: cAMP ELISA Kit (Chemiluminescent)
2. STA-505: cGMP ELISA Kit (Colorimetric)
3. STA-506: cGMP ELISA Kit (Chemiluminescent)

## **Kit Components**

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250001): One strip well 96-well plate.
2. cAMP Standard (Part No. 250002): One 100  $\mu$ L vial provided at 10 mM.
3. Rabbit Anti-cAMP Polyclonal Antibody (Part No. 250003): One 15  $\mu$ L vial.
4. Peroxidase cAMP Tracer Conjugate (Part No. 250004): One 30  $\mu$ L vial.
5. Assay Diluent (Part No. 250005): One 25 mL bottle.
6. Lysis Buffer (Part No. 250006): One 50 mL bottle.
7. 10X Wash Buffer (Part No. 250007): One 50 mL bottle.
8. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
9. Stop Solution (Part. No. 310808): One 12 mL bottle.

## **Materials Not Supplied**

1. Orbital plate shaker
2. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
3. 50  $\mu$ L to 300  $\mu$ L adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
6. (optional) Acetylation reagents: acetic anhydride and triethylamine

## **Storage**

Store kit components at 4°C until their expiration dates. For longer term use, store the Rabbit Anti-cAMP Polyclonal Antibody at -20°C.

## **Preparation of Reagents**

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Rabbit Anti-cAMP Polyclonal Antibody: Immediately before use dilute the Rabbit Anti-cAMP Antibody 1:500 with Assay Diluent. Do not store diluted solutions.
- Peroxidase cAMP Tracer Conjugate: Immediately before use dilute the Peroxidase cAMP Tracer Conjugate 1:100 with Assay Diluent. Do not store diluted solutions.

## **Preparation of cAMP Standards**

1. Freshly prepare a dilution series of cAMP Standard in the concentration range of 100  $\mu$ M – 100 pM by diluting the cAMP Standard in Lysis Buffer (Table 1).

Standard Tubes	cAMP Standard ( $\mu$ L)	Lysis Buffer ( $\mu$ L)	cAMP Concentration
1	10	990	100 $\mu$ M
2	20 of Tube #1	180	10 $\mu$ M
3	20 of Tube #2	180	1 $\mu$ M
4	20 of Tube #3	180	100 nM
5	20 of Tube #4	180	10 nM
6	20 of Tube #5	180	1 nM
7	20 of Tube #6	180	100 pM
8	0	180	0

**Table 1. Preparation of cAMP Standard Curve**

## **Preparation of Samples**

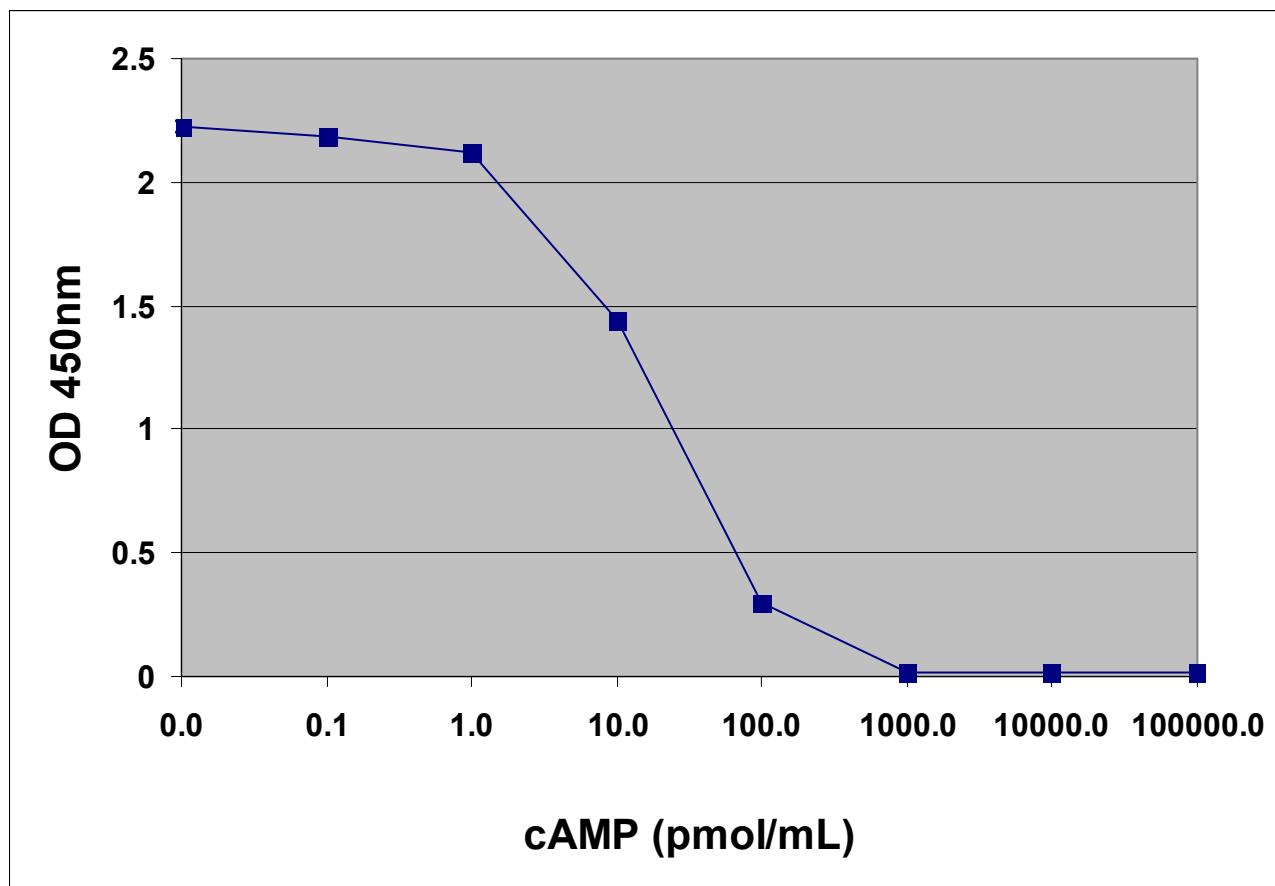
1. **Urine, Plasma and Culture Medium Samples:** Urine and plasma may be tested directly with 1:200 to 1:1000 dilutions in Lysis Buffer. Culture medium can also be tested with 1:10 to 1:200 dilutions in Lysis Buffer.  
**Note:** RPMI medium may contain >350 fmol/ $\mu$ L cAMP).
2. **Cell Samples:** Aspirate medium. Add 1 ml of Lysis Buffer for every 35  $\text{cm}^2$  of surface area. Incubate at 4  $^{\circ}$ C for 20 minutes. Scrape cells off the surface with a cell scraper. Dissociate sample by pipetting up and down until suspension is homogeneous. Transfer to a centrifuge tube and centrifuge at top speed for 10 min. The supernatant can be assayed directly. Protein concentration >1 mg/ml is recommended for reproducible results.
3. **Tissue Samples:** Cyclic nucleotides may be metabolized quickly in tissue, so it is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen). Weigh the frozen tissue and add 5-10  $\mu$ L of Lysis Buffer per mg of tissue. Homogenize the sample on ice using a Polytron-type homogenizer. Spin at top speed for 5 min and collect the supernatant. The supernatant may be assayed directly.

## **Assay Protocol**

1. Prepare and mix all reagents thoroughly before use.
2. Each cAMP sample, cAMP Standard, and blank should be assayed in duplicate.
3. Add 50  $\mu$ L of cAMP sample or standard to the Goat Anti-Rabbit Antibody Coated Plate.
4. Add 25  $\mu$ L of diluted Peroxidase cAMP Tracer Conjugate (see Preparation of Reagents Section) to each tested well.
5. Add 50  $\mu$ L of diluted Rabbit Anti-cAMP Polyclonal Antibody (see Preparation of Reagents Section) to each tested well.
6. Cover with a Plate Cover and incubate at room temperature for 2 hours with shaking.
7. Remove Plate Cover and empty wells. Wash microwell strips 5 times with 250  $\mu$ L 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
8. Warm Substrate Solution to room temperature. Add 100  $\mu$ L of Substrate Solution to each well, including the blank wells. Incubate at room temperature for 5-20 minutes on an orbital shaker.
9. Stop the enzyme reaction by adding 100  $\mu$ L of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

## **Example of Results**

The following figures demonstrate typical cAMP ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: cAMP ELISA Standard Curve**

### **Cross reactivity of cAMP ELISA Kit**

<u>Compounds</u>	<u>Cross Reactivity</u>
cAMP	100%
cGMP	<0.1%
AMP	<0.01%
ADP	<0.01%
ATP	<0.01%
GMP	<0.01%
GTP	<0.01%
CTP	<0.01%

## **References**

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## **Warranty**

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