Product Manual

cAMP ELISA Kit (Chemiluminescent)

Catalog Numbers

STA-501	96 assays
STA-501-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

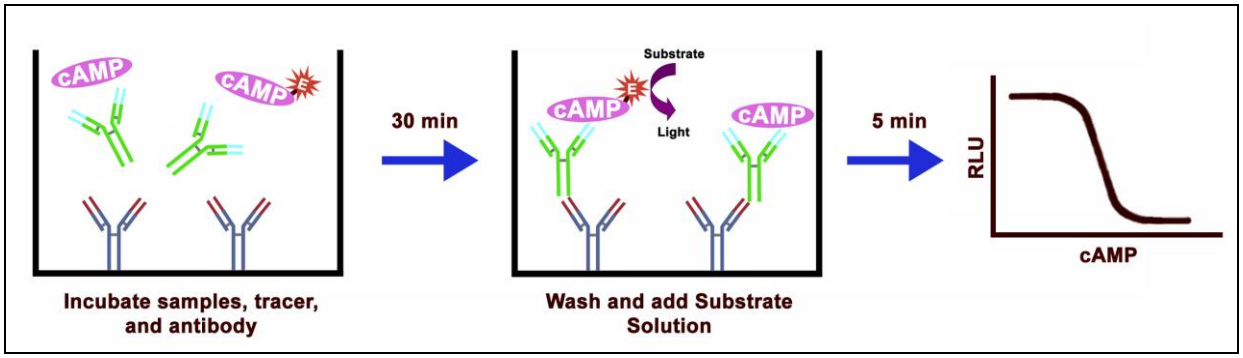
Introduction

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 Ca^{2+} permeability, can alter the levels of intracellular 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. Samples containing low cAMP levels may be acetylated (reagents provided) for increased sensitivity. Under non-acetylated conditions, the kit has a detection range of 1 to 1000 pmol/mL cAMP; however, under acetylated conditions, the sensitivity is enhanced (approx 100X) to a detection range of 10-2500 fmol/mL.

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 Chemiluminescent Reagent. The light product formed is inversely proportional to the amount of cAMP present in the sample. This reaction is then measured in a plate luminometer. A standard curve is prepared from cAMP Standard and sample concentration is then determined.



Related Products

1. STA-415: ROCK Activity Immunoblot Kit
2. STA-416: 96-Well ROCK Activity Assay Kit
3. STA-500: cAMP ELISA Kit (Colorimetric)

Kit Components (shipped at room temperature)

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250101): One strip well 96-well plate.
2. cAMP Standard (Part No. 250002): One 100 µL vial provided at 10 mM.
3. Rabbit Anti-cAMP Polyclonal Antibody (Part No. 250003): One 15 µL vial.
4. Peroxidase cAMP Tracer Conjugate (Part No. 250004): One 30 µL 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. Triethylamine (Part No. 250008): One 2 mL amber bottle.
9. Acetic Anhydride (Part No. 250009): One 1 mL amber bottle.
10. Chemiluminescent Reagent A (Part No. 250102): One 6 mL amber bottle.
11. Chemiluminescent Reagent B (Part. No. 250103): One 6 mL bottle.

Materials Not Supplied

1. Orbital plate shaker
2. 96-well Plate Luminometer
3. Glass or polypropylene tubes for acetylated samples and standards

Storage

Store both the Rabbit Anti-cAMP Polyclonal Antibody and the Peroxidase cAMP Tracer Conjugate at -20ºC. Store all other kit components at 4ºC.

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.

- Chemiluminescent Reagent: Immediately before use, mix equal volumes of Chemiluminescent Reagent A with Chemiluminescent Reagent B. Do not store diluted solutions.
- Acetylation Reagent: Preparation of the Acetylation Reagent should be done in glass tubes and in a fume hood. The Acetylation Reagent is made by mixing Acetic Anhydride with Triethylamine at a 1:2 ratio (example: 0.5 mL Acetic Anhydride + 1 mL Triethylamine). Use the reagent within 60 minutes of preparation.

  Caution: The components of this reagent are known to be caustic, corrosive, flammable, and lachrymators. Use appropriate protection when handling.

Preparation of cAMP Standards (Non-Acetylated Version)

1. Thaw the cAMP Standard at room temperature and mix thoroughly by pipetting (cAMP can precipitate when frozen but will redissolve when mixed well). Freshly prepare a dilution series of cAMP Standard in the concentration range of 100 µM – 100 pM by diluting the cAMP Standard in Lysis Buffer (Table 1).

Standard Tubes	cAMP Standard (µL)	Lysis Buffer (µL)	cAMP Concentration (nM)
1	10	990	100,000
2	20 of Tube #1	180	10,000
3	20 of Tube #2	180	1000
4	20 of Tube #3	180	100
5	20 of Tube #4	180	10
6	20 of Tube #5	180	1
7	20 of Tube #6	180	0.1
8	0	180	0

Table 1. Preparation of Non-Acetylated cAMP Standard Curve

Preparation of Samples (Non-Acetylated Version)

- Urine, Serum, Plasma and Culture Medium Samples: Urine, serum 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/µL cAMP).

- Cell Samples: Aspirate medium. Add 1 ml of Lysis Buffer for every 35 cm^2 of surface area. Incubate at 4ºC 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.

- 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 µL 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.

Preparation of cAMP Standards (Acetylated Version)

Note: Samples containing low cAMP levels may be acetylated for increased sensitivity (approx 100-fold), although overall assay values will be lowered 2-5-fold.

1. Thaw the cAMP Standard at room temperature and mix thoroughly by pipetting (cAMP can precipitate when frozen but will redissolve when mixed well). In glass or polypropylene tubes, freshly prepare a dilution series of cAMP Standard in the concentration range of 10 nM – 2.4 pM by diluting the cAMP Standard in Lysis Buffer (Table 2).

   Note: The kit cAMP Standard, provided at 10 mM, must first be aggressively diluted to achieve the desired range. A series of 1:100 dilutions are suggested (denoted Stock A and B). Stock A and B are not to be included in the standard curve; only tubes 1-8 should be transferred.

Standard Tubes	cAMP Standard (µL)	Lysis Buffer (µL)	Final cAMP Concentration (nM)
Stock A	10 of cAMP Standard (10 mM)	990	100,000
Stock B	10 of Stock A	990	1000
1	10 of Stock B	990	10
2	100 of Tube #1	300	2.5
3	100 of Tube #2	300	0.625
4	100 of Tube #3	300	0.156
5	100 of Tube #4	300	0.039
6	100 of Tube #5	300	0.010
7	100 of Tube #6	300	0.0025
8	0	300	0

Table 2. Preparation of Acetylated cAMP Standard Curve

2. In the hood, transfer 200 µL of tubes 1-8 to new tubes and acetylate each by adding 10 µL of Acetylation Reagent (see Preparation of Reagents). Mix well and use within 30 minutes.

Preparation of Samples (Acetylated Version)

Note: Samples containing low cAMP levels may be acetylated for increased sensitivity (approx 100-fold), although overall assay values will be lowered 2-5 fold.

- Urine, Serum, Plasma and Culture Medium Samples: Urine, serum 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. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.
  Note: RPMI medium may contain >350 fmol/µL cAMP).

- Cell Samples: Aspirate medium. Add 1 ml of Lysis Buffer for every 35 cm^2 of surface area. Incubate at 4ºC 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. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.

- 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 µL 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. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.

Assay Protocol

Important Note: Add reagents to the plate gently using a multichannel pipette. To avoid the creation of bubbles in the well, do not mix by pipetting.

1. Prepare and mix all reagents thoroughly before use.
2. Each cAMP sample, cAMP Standard, and blank should be assayed in duplicate.
   Note: cAMP samples must be compared with corresponding standards (i.e. acetylated samples compared with acetylated standards; non-acetylated samples with non-acetylated standards).
3. Add 50 µL of cAMP sample or standard (acetylated or non-acetylated) to the Goat Anti-Rabbit Antibody Coated Plate.
4. Add 25 µL of diluted Peroxidase cAMP Tracer Conjugate (see Preparation of Reagents Section) to each tested well.
5. Add 50 µL 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 30 minutes with shaking.
7. Remove Plate Cover and empty wells. Wash microwell strips 5 times with 250 µL 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. Add 100 µL of Chemiluminescent Reagent (see Preparation of Reagents Section) to each well, including the blank wells. Incubate at room temperature for 5 minutes on an orbital shaker.
9. Read the luminescence of each microwell on a plate luminometer.

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.

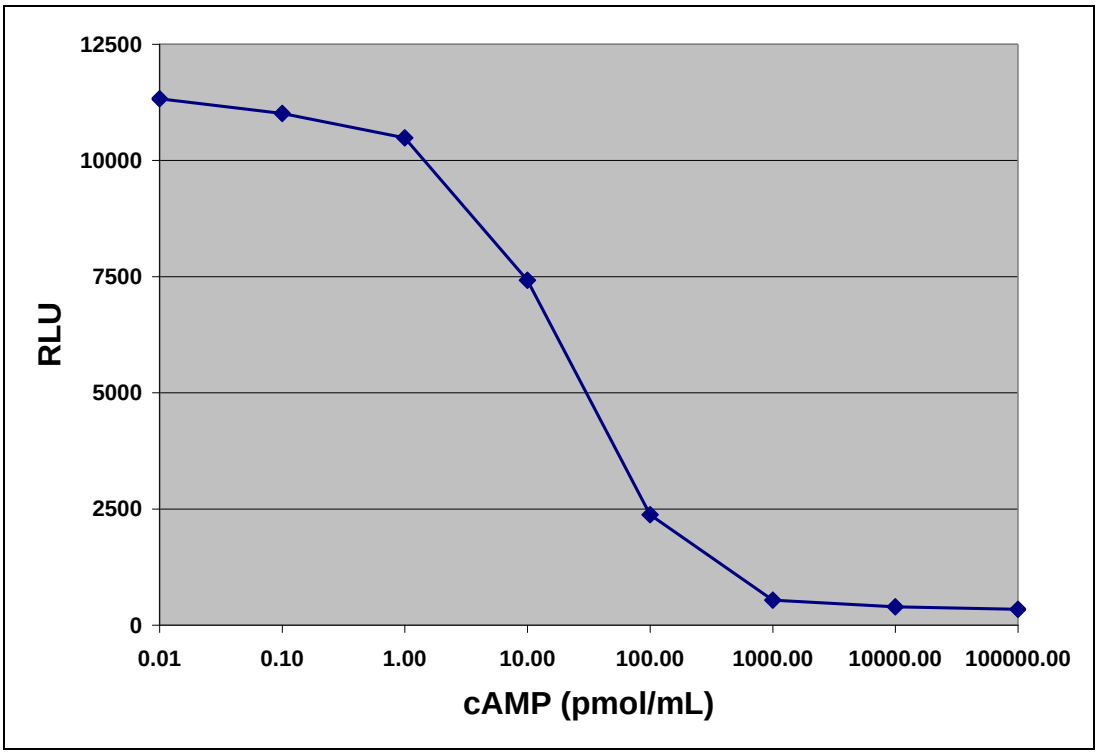


Figure 1: cAMP ELISA Standard Curve (Non-Acetylated Version)

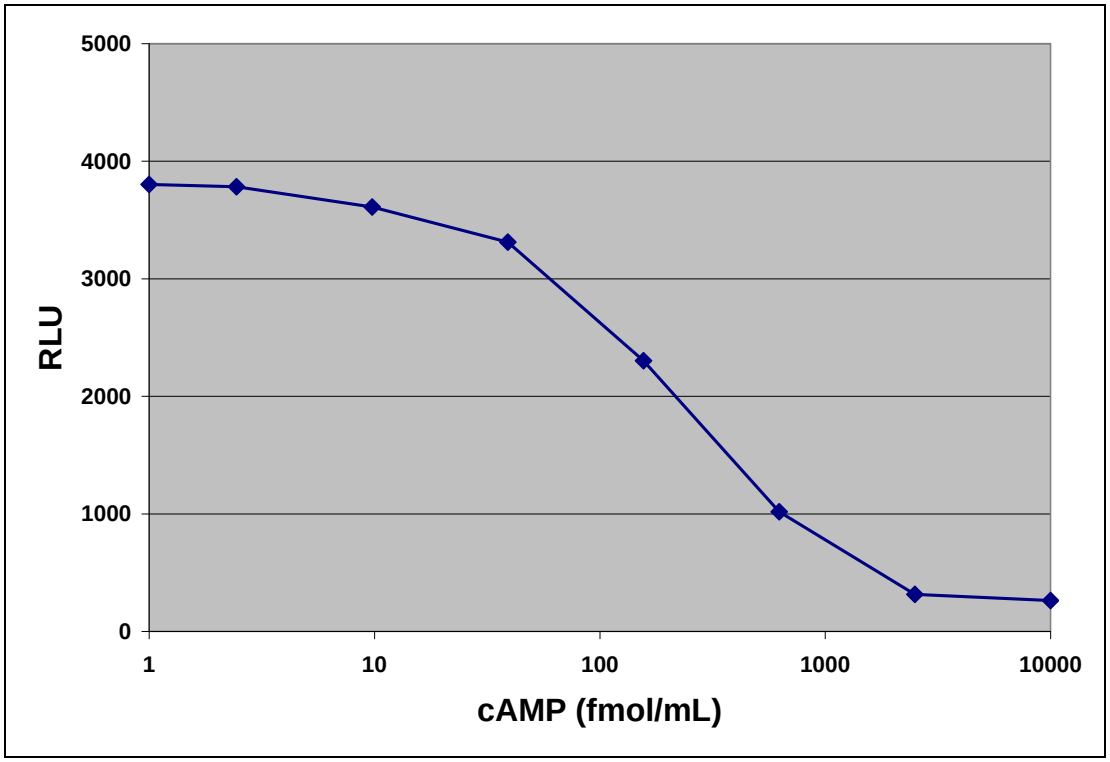


Figure 2: cAMP ELISA Standard Curve (Acetylated Version)

Cross reactivity of cAMP ELISA Kit

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

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Recent Product Citations

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