cAMP ELISA Kit (Chemiluminescent)

Competitive Enzyme Immunoassay for the quantification of Cyclic AMP in serum, plasma, urine, saliva, cell lysate and cell culture supernatants.

Catalog number: ARG82211

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INTRODUCTION

Cyclic adenosine monophosphate (cAMP, cyclic AMP, or 3',5'-cyclic adenosine monophosphate) is a second messenger important in many biological processes. cAMP is a derivative of adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway.

Since cyclic AMP is a second messenger and plays vital role in cell signalling, it has been implicated in various disorders but not restricted to the roles given below:

Role in human carcinoma: Some research has suggested that a deregulation of cAMP pathways and an aberrant activation of cAMP-controlled genes is linked to the growth of some cancers.

Role in prefrontal cortex disorders:

Recent research suggests that cAMP affects the function of higher-order thinking in the prefrontal cortex through its regulation of ion channels called hyperpolarization-activated cyclic nucleotide-gated channels (HCN). When cAMP stimulates the HCN, the channels open, closing the brain cell to communication and thus interfering with the function of the prefrontal cortex. This research, especially the cognitive deficits in age-related illnesses and ADHD, is of interest to researchers studying the brain. [provide from Wikipedia]

PRINCIPLE OF THE ASSAY

This is a Competitive Enzyme Immunoassay for the quantification cyclic adenosine monophosphate (cAMP) in serum, plasma, urine, saliva, cell lysate and cell culture supernatants samples.

A Goat anti rabbit IgG antibody has been pre-coated onto a microtiter plate. Then HRP-congugated cAMP, rabbit anti-cAMP antibody, standards or samples are added in the wells. The cAMP antibody in the kit will be competitively bound by HRP-congugated cAMP and cAMP in standards or samples. After washing away any unbound antibody-enzyme reagent, a Chemiluminescent Reagent substrate solution is added to the wells and light develops in inverse-proportion to the amount of cAMP present in samples or standards. This reaction is then measured in a plate luminometer. The concentration of cAMP in the sample is then determined by comparing the RLU of samples to the standard curve.

The cAMP in the samples or cAMP in the standards compete with the HRP-conjugated cAMP for antibody binding site. High cAMP content in a sample or high concentration cAMP standard results in less HRP-cAMP-antibody binding complex on the plate, resulting in a low signal. Conversely, low cAMP content in a sample or low concentration cAMP standard result in most HRP-cAMP binding to the cAMP antibody on the plate, producing a higher signal.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all kit components at 4°C. For longer term use, store the Rabbit Anti-cAMP Antibody at -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
Secondary antibody coated microplate	1 strips X 96 wells	4°C
cAMP Standard (10 mM)	100 µl	4°C
500X rabbit Anti-cAMP Antibody	15 µl	-20°C
cAMP Conjugate concentrate	30 µl	4°C
10X Wash Buffer	50 ml	4°C
Lysis Buffer	50 ml (Ready-to-use)	4°C
Assay Diluent	25 ml (Ready-to-use)	4°C
Triethylamine	2ml	4°C (Protect from light)
Acetic Anhydride	1ml	4°C (Protect from light)
Chemiluminescent Reagent A	6ml (Ready-to-use)	4°C (Protect from light)
Chemiluminescent Reagent B	6ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Plate Luminometer
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- (Optional) lysis buffer without BSA: 50 mM Tris buffer (pH 6.0) contains 2% Triton X-100, 0.01% Thimerosal (optional).
- Microplate shaker.
- Glass or polypropylene tubes for acetylated samples and standards
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all other kit components at 4°C. For longer term use, store the Rabbit Anti-cAMP Antibody at -20°C. Use the kit before expiration date.
- Briefly mix and spin down the components before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: Haemolytic and especially lipemic samples should not be used with this assay.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C or below.

Cell lysate: Aspirate medium. Wash cells with PBS 1-2 times. Add 1 ml of Lysis Buffer for every 35 cm^2 of surface area. Incubate at 4°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 at 4°C. Collect the supernatant and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Protein concentration >1 mg/ml is recommended for reproducible results.

Tissue lysate: 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 at 4°C. Collect the supernatant and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The lysis buffer included with this kit contains 0.1% BSA and it is an interference for protein assay. So for measuring protein concentration, we suggested lysing samples with lysis buffer without BSA: 50 mM Tris buffer (pH 6.0) contains 2% Triton X-100, 0.01% Thimerosal (optional).

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. (e.g. Example: Add 50 ml of 10X wash buffer to 450 mL of distilled water, mix well.) Storage the diluted wash buffer at 2-8°C.
- Anti-cAMP Antibody: Dilute the antibody immediately before use, dilute the 500X Anti-cAMP antibody into Assay Diluent to yield 1X antibody working solutions. Do not store diluted solutions.
- 1X cAMP Conjugate: Dilute reagent immediately before use, diluting the cAMP Conjugate concentrate at 100X dilution with Assay Diluent to yield 1X cAMP Conjugate working solution. (e.g. Example: Add 30 µl of cAMP Conjugate concentrate to 2.970 ml of Assay Diluent)
- Chemiluminescent Reagent Mixture: 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.
- Sample:

  - Urine, serum and plasma may be diluted at 1:200 to 1:1000 dilutions with Lysis Buffer before assay.

  - Culture medium can also be tested with 1:10 to 1:200 dilutions in Lysis Buffer. (RPMI medium may contain >350 fmol/µL cAMP)

- Cell/tissue lysate may be tested directly after collection or diluted with Lysis Buffer at appropriate dilution.

- If the initial assay found samples contain cAMP higher than the highest standard, the samples can be diluted with Lysis Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

- It is recommended to do pre-test to determine the suitable dilution factor.

- **cAMP standard:**

Standard preparation for Non-Acetylated Version

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 series dilution of cAMP standards with Lysis Buffer. The Lysis Buffer serves as zero standard (0 µM), and the rest of the standard serial dilution can be diluted with Lysis Buffer as according to the suggested concentration table below:

Standard No	cAMP concentration	Lysis Buffer (µl)	Standards (µl)
S1	100 µM	990 µl	10 µl (10 mM stock)
S2	10 µM	180 µl	20 µl (S1)
S3	1 µM	180 µl	20 µl (S2)
S4	0.1 µM (100 nM)	180 µl	20 µl (S3)
S5	0.01 µM (10 nM)	180 µl	20 µl (S4)
S6	0.001 µM (1 nM)	180 µl	20 µl (S5)
S7	0.0001 µM (100 pM)	180 µl	20 µl (S6)
S0	0	180 µl	0 µl

Note: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately. S0 serves as Total binding.

Standard preparation for Acetylated Version

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

Freshly prepare a series dilution of cAMP standards with Lysis Buffer. Thaw the cAMP Standard stock at room temperature and mix thoroughly by pipetting (cAMP can precipitate when frozen but will redissolve when mixed well). Freshly prepare cAMP standards with Lysis Buffer. Dilute the 10 mM standard stock at 100X dilution with Lysis Buffer to yield a Stock-1 concentration of 100 µM. Further dilute the Stock-I at 100X dilution with Lysis Buffer to yield a Stock-2 concentration of 1 µM. The Lysis Buffer serves as zero standard (0 µM), and the rest of the standard serial dilution can be diluted with Lysis Buffer as according to the suggested concentration table below:

Standard No	cAMP concentration	Lysis Buffer (µl)	Standards (µl)
Stock-1	100 µM	990 µl	10 µl (10 mM stock)
Stock-2	1 µM	990 µl	10 µl (Stock-1)
S1	10 nM	990 µl	10 µl (Stock-2)
S2	2.5 nM	300 µl	100 µl (S1)
S3	625 pM	300 µl	100 µl (S2)
S4	156 pM	300 µl	100 µl (S3)
S5	39 pM	300 µl	100 µl (S4)
S6	9.8 pM	300 µl	100 µl (S5)
S7	2.4 pM	300 µl	100 µl (S6)
S0	0	300 µl	0 µl

Note: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately. S0 serves as Total binding.

- **Sample and Standard Acetylation:** In a fume hood, transfer 200 µL of Srandard S0, S1-S7 and diluted samples to new tubes and acetylate each by adding 10 µL of Acetylation Reagent (Acetic Anhydride / Triethylamine mixture, refer the detail at above). Mix well and use within 30 minutes.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. When diluting samples and reagents, they must be mixed completely and evenly. Add reagents to the plate gently using a multichannel pipette. To avoid the creation of bubbles in the well, do not mix by pipetting. Standard detection curve should be prepared for each experiment. Standards, samples and controls should be assayed in duplicates. (Note: cAMP samples must be compared with corresponding standards. i.e. if samples were acetylated, the standards should also be acetylated. Standards and samples should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl of the prepared cAMP standards and samples** into the appropriate wells of the secondary antibody coated plate.
3. Add **25 µl of the diluted 1X cAMP Conjugate** to each well.
4. Add **50 µl of the diluted 1X Anti-cAMP Antibody** working solution to each well, cover the plate and incubate for **30 min at room temperature** on a microplate shaker.
5. Remove the Plate Cover and aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher.

Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

6. Add 100 µl of Chemiluminescent Reagent Mixture solution into each well. Incubate for 5 min at RT on a microplate shaker. Avoid exposure to light.
7. Read the luminescence of each microwell on a plate luminometer immediately.

CALCULATION OF RESULTS

1. Calculate the average RLU (relative light units) values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean RLU obtained from each standard against its concentration with RLU value on the vertical (Y) axis and concentration on the horizontal (X) axis.

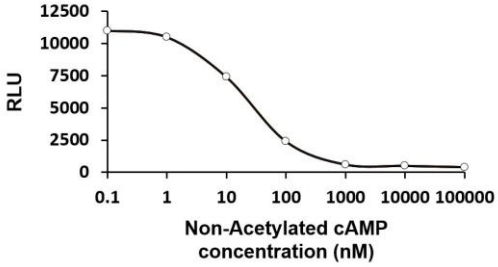
3. Using the mean RLU value for each sample determine the corresponding concentration from the standard curve.

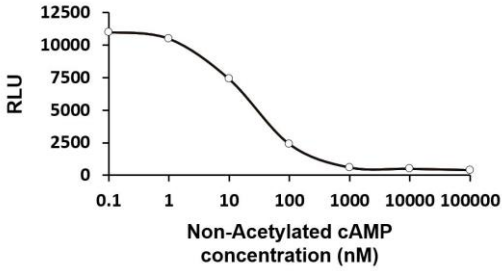
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.





QUALITY ASSURANCE

Sensitivity

1 nM (Non-Acetylated cAMP)

10 pM (Acetylated cAMP)

Assay Range

0.1- 100,000 nM (Non-Acetylated cAMP)

2.4- 10,000 pM (Acetylated cAMP)

Detection Range

1-1000 nM (Non-Acetylated cAMP)

10-2500 pM (Acetylated cAMP)

Specificity

cAMP: 100%

cGMP: < 0.1%

AMP, ADP, ATP, GMP, GTP and CTP: < 0.01%