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ab65355 cAMP Direct Immunoassay Kit

View cAMP Direct Immunoassay Kit datasheet:
www.abcam.com/ab65355
(use www.abcam.cn/ab65355 for China, or www.abcam.co.jp/ab65355 for Japan)

For the measurement of cAMP in Tissue Extracts, Cell Lysate, Cell culture media, Urine, Serum and other biological fluids.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

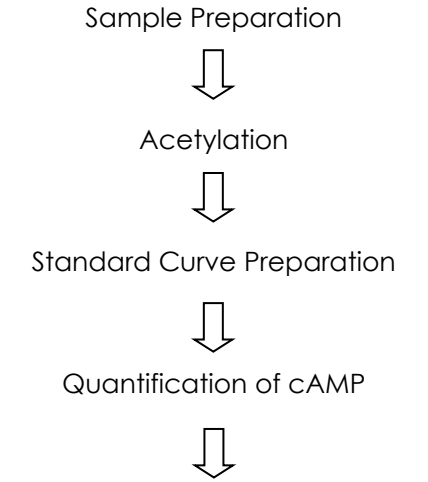
Table of Contents

1. Overview

Abcam cAMP (Adenosine 3', 5'-cyclic monophosphate) Assay Kit provides a direct competitive immunoassay for sensitive and quantitative measurement of cAMP level in Tissue Extracts, Cell Lysates, Cell culture media, Urine, Serum and other biological fluids.

The kit includes a recombinant Protein G coated 96-well plate that facilitate binding of cAMP polyclonal antibody on to the plate. cAMP-HRP conjugate directly competes with cAMP in the sample for binding to cAMP antibody bound on the plate. After incubation and washing, the amount of cAMP-HRP bound to the plate can easily be measured by reading HRP activity at OD450 nm. The intensity of OD450 nm is inversely proportional to the concentration of cAMP in samples.

The kit provides a new acetylation procedure for improving detection sensitivity significantly. The kit can detect ~0.1-10 pmol/50 µl (per well) (or ~0.002-0.2 µM) cAMP samples.



2. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Thaw cAMP Standard (aliquot if necessary), Rabbit Anti-cAMP Ab/Rabbit Anti-cAMP pAb (aliquot if necessary) and cAMP-HRP; get equipment ready.
- Prepare cAMP standard and sample dilutions, label and add 100 µLin each microcentrifuge tube.
- Add 50 µL Neutralization Buffer III/Neutralizing buffer to each Standard and test sample tube.
- Prepare Acetylating Reagent Mix and add 5 µL in each standard and sample tube.
- Add 845 µL Assay Buffer XXXVI/Assay buffer in each tube, mix well.
- Transfer 50 µL acetylated cAMP Standard/Standard cAMP and test samples into provided Protein G coated 96-well plate.
- Add 10 µL Rabbit Anti-cAMP Ab/Rabbit Anti-cAMP pAb in each standard and test sample well excluding 0_background, add 10 µL Assay Buffer XXXVI/1X Assay Buffer for background reading. Incubate for 1 hour.
- Add 10 µL cAMP-HRP in each well, mix and incubate for 1 hour with gentle agitation.
- Add 100 µL TMB Substrate I/HRP developer, incubate for 1 hour at room temperature.
- Add stop solution 100 µL 1M HCl (sample color should change from blue to yellow).
- Read the plate at OD450 nm.

3. Materials Supplied and Storage

Store kit at -20°C immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 1-2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer XXXVI/10X cAMP Assay Buffer	25 mL	-20°C	+4°C
cAMP Standard/Standard cAMP (10 nmol)	1 vial	-20°C	-20°C
Neutralization Buffer III/Neutralizing Buffer	7.5 mL	-20°C	+4°C
Acetylating Reagent A	0.75 mL	-20°C	+4°C
Acetylating Reagent B	1.5 mL	-20°C	+4°C
Rabbit Anti-cAMP Ab/Rabbit Anti-cAMP pAb	1 vial	-20°C	-20°C
cAMP-HRP	1 vial	-20°C	-20°C
TMB Substrate I/HRP Developer	10 mL	-20°C	+4°C
Protein G Coated Plate	1 each	-20°C	-20°C

4. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 0.1 M and 1 M HCl
- Dounce homogenizer (if using tissue)
- Microplate reader capable of measuring absorbance at OD450 nm.

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Assay Buffer XXXVI/10X cAMP Assay Buffer

Dilute the 10X Assay Buffer XXXVI/cAMP Assay Buffer to 1X Assay Buffer XXXVI/Assay Buffer with MilliQ water (add 225 mL MilliQ water to 10X buffer).

6.2 cAMP Standard/Standard cAMP (10 nmol)

Reconstitute the cAMP Standard/Standard cAMP (pellet may not be visible) in 1 mL of 0.1M HCl and vortex for 10 seconds to generate a
10 pmol/µL cAMP standard stock solution. Store at -20°C, use within 2 months.

6.3 Acetylating Reagents (A + B)

Ready to use as supplied. Warm to room temperature prior to use. The Acetylating Reagent B is very volatile hence, vial must be tightly capped and stored only at +4°C.

6.4 Rabbit Anti-cAMP Ab/Rabbit Anti-cAMP pAb

Dilute the Rabbit Anti-cAMP Ab/rabbit anti-cAMP polyclonal antibody in 1.1 mL of the 1X Assay Buffer XXXVI/Assay Buffer (step 6.1) to create a stock solution. Aliquot antibody so that you have enough to perform the desired number of assays.

6.5 cAMP-HRP

Dilute the cAMP-HRP conjugate in 1.1 mL of distilled/ Milli-Q water to create a stock solution. Aliquot conjugate so that you have enough to perform the desired number of assays.

6.6 TMB Substrate I/HRP Developer

Ready to use as supplied. Warm to room temperature prior to use.

6.7 Protein G Coated Plate

Store unused Protein G coated strips with desiccants at -20°C. After opening, the strips are stable for up to 1 month.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute 25 µL of the 10 pmol/µL cAMP standard stock solution (from Section 6.2) with 975 µL of 0.1M HCl to generate 0.25 pmol/µL cAMP working solution. The diluted cAMP should be freshly made each time, use within 1 hour.
2. In microcentrifuge tubes, perform serial dilutions as follows, using the cAMP 0.25 pmol/µL solution.
3. Discard 100 µL from 0.039 pmol tube.
4. Follow the procedure onward step 9.1.

Standard	cAMP Standard (µL)	0.1 M HCl (µL)	Final volume in well (µL)	End amount of cAMP standard in well (pmol/50 µL)
1	200 µL of cAMP 0.25 pmol/µL solution	0	100	1.25
2	100 µL of std 1	100	100	0.625
3	100 µL of std 2	100	100	0.3125
4	100 µL of std 3	100	100	0.156
5	100 µL of std 4	100	100	0.078
6	100 µL of std 5	100	100	0.039
7	0	100	100	0
8	0	100	100	0_sample background

Prepare enough standard to set up duplicate readings (2 x 100 µL).

The dilutions for the standard measurement should be used within 1 hour.

8. Sample Preparation

General sample information:

We recommend performing several dilutions of your unknown sample to ensure the readings are within the standard value range (0.02 – 2 µM cAMP range).

We recommend that you use fresh samples for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.

Make sure all buffers and solutions are at room temperature, and keep cAMP standard, anti-cAMP pAb and cAMP-HRP on ice.

8.1 Tissue samples:

1. Weigh the frozen tissue and add 5X– 10X volume of 0.1M HCl. Initial recommendation 100 mg tissue.
2. Homogenize the sample on ice using a Polytron-type homogenizer.
3. Centrifuge at top speed for 5 min and collect the supernatant. Keep on ice for immediate use or store at -80°C.
4. Use 100 µL of sample per test. If volume needed is <100 µL, bring it up to 100 µL with 0.1M HCl.
5. Protein concentration of the samples should be ≥ 1 mg/mL for reproducible results.

8.2 Cell Samples:

1. Harvest the number of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).
2. Add 282 µL of 0.1M HCl in 35 mm plate (Surface area = 10 cm^2) or 1 mL of 0.1M HCl for every 35 cm^2 of surface area, e.g. for a 150 mm plate (Surface area= 176.7 cm^2), add 5 mL of 0.1 N HCl and incubate at room temperature for 20 min.
3. Scrape cells off the plate surface using a cell scraper.
4. Dissociate sample by pipetting up and down until suspension is homogeneous.

5. Centrifuge suspension at top speed for 10 min to remove any insoluble material
6. Protein concentration of the samples should be ≥ 1 mg/mL for reproducible results.

8.3 Biological fluid (Urine, Plasma and Culture Medium Samples):

1. Urine and Plasma may be tested directly at a dilution of 1:20 – 1:100 in 0.1M HCl. Store diluted samples at -80°C.
2. Culture media can be tested directly at a dilution of 1:10 – 1:200 in 0.1M HCl.

Δ Note: RPMI media may contain >350 fmol/µL cAMP. Take this into consideration when preparing samples from RPMI medium.

Δ Note: Phosphodiesterases (PDEs) and large number of immunoglobulins (IgGs) present in samples may degrade cAMP and can interfere with the assay. Diluting samples with 0.1M HCl generally inactivates PDEs and lowers the concentration of IgGs, making the samples suitable for the assay. Dilute to ~0.1- 10 pmol/well (0.02-2 µM) cAMP range.

Δ Note: To determine interfering substances in samples, you may make two different dilutions. If the two different dilutions of sample show good correlation in the final calculated cAMP concentrations, purification is not required. Otherwise, remove any interfering enzymes/ proteins from samples using 5% TCA Deproteinizing sample preparation kit (ab204708) or Spin Columns 10 kDa molecular weight cut off microcentrifuge filters (ab93349).

Δ Note: Organic solvents in samples may also interfere with the assay, they may need to be removed prior to the assay.

9. Assay Procedure

– Assay all standards, controls and samples in duplicate.

9.1 cAMP Assay

1. Prepare microcentrifuge tubes for test samples: add 100 µL each test sample per tube. We suggest using different dilutions for each sample (diluted in 0.1M HCl).
2. Add 50 µL of Neutralization Buffer III/Neutralizing Buffer to each tube (all standards cAMP and test samples, in duplicate). Mix quickly by inversion.

Acetylating Reaction mix:

3. Mix 1X volume of Acetylating Reagent A with 2X volumes of Acetylating Reagent B in an Eppendorf tube. Prepare enough for the experiment (you will need 5 µL for each sample and standard tubes). Use within 1 hour.
4. Add 5 µL of the Acetylating Reagent Mix directly into each test solution (all standards and samples), vortex immediately for 2-3 secs following each addition without delay, one tube at a time, and incubate at room temperature for 10 min to acetylate cAMP.
5. Add 845 µL 1X Assay Buffer XXXVI/Assay Buffer into each tube to dilute the acetylation reagents, mix well.
6. The acetylated standard and samples are ready for quantification.

Δ Note: If cAMP level in your samples is very low, the acetylation reagents can be dried after step 4 without diluting in step 5. Reconstitute then with 50 – 100 µL of 1X Assay Buffer XXXVI/Assay Buffer.

Δ Note: The procedure described above includes an acetylation step which makes the cAMP assay much more sensitive by avoiding interferences of many components in samples. However, for routine assay of well-known samples, non-acetylation procedure may also be used, just skip the acetylation steps (Step 9.1.3 and 9.1.4).

9.2 cAMP Quantification:

1. Add 50 µL of the acetylated cAMP Standard/Standard cAMP and test samples from Step 9.1.6 to the Protein G coated 96-well plate.
2. Perform duplicate readings of each standard and test sample to ensure consistency on the reading.
3. Add 10 µL of the reconstituted cAMP antibody/well to the cAMP Standard/standard cAMP and sample wells, except on the standard 8 (0_BACKGROUND). In the well 0_BACKGROUND, add 10 µL 1X Assay Buffer XXXVI/Assay Buffer for background reading).
4. Incubate for 1 hr at room temperature with gentle agitation.
5. Add 10 µL of cAMP-HRP to each well, mix well by pipetting up and down and incubate for 1 hr at room temperature with agitation.
6. Gently, wash 5 times with 200 µL 1X Assay Buffer XXXVI/Assay Buffer each wash.
7. Empty the wells completely by tapping the plate on a new paper towel after each wash step.
8. Add 100 µl of TMB Substrate I/HRP developer and read the OD 650 nm at kinetic mode for 1 hour at room temperature with agitation.
9. Note: You should stop the reaction when the OD 650 nm of 0 pmol cAMP reaches 0.8-1.0.
10. Stop the reaction by adding 100 µL of 1M HCl to each well (sample color should change from blue to yellow).
11. Read the plate at OD450 nm.

Δ Note: The OD450 nm readings may vary significantly among experiments depending on lot numbers, kit storage and experimental conditions; therefore, samples and standard curve must be performed at the same time and using the same kit reagents.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, background control and sample.
2. Subtract the mean value of the blank (Standard #8) from all standards, background controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of cAMP.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

$$y = mx + b$$

6. Apply the corrected sample OD reading to the standard curve to get cAMP amount in the sample wells.

$$Sa = \left(\frac{Corrected\ absorbance - (y\ intercept)}{Slope}\right)$$

7. Concentration of cAMP in the test samples is calculated as

$$\text{cAMP concentration} = \frac{\text{Sa}}{\text{Sv}} * \text{D}$$

Where:

Sa = cAMP amount (pmol) from the Standard Curve.

Sv = ample volume (µl) added into the assay wells

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

In case of spiked samples use the following equation, wherever required;

8. Using **spiked samples**, correct for any sample matrix interference by subtracting the sample reading from the spiked sample reading. This equation allows you to measure the cAMP analyte concentration in your sample when matrix interference is significant.

$$B = \left(\frac{(ODsample\ corrected)}{(ODspiked\ corrected) - (ODsample\ corrected)}\right) * Analyte\ Spike\ (pmol/nmol)$$

Where:

B = Analyte amount in sample well (pmol/nmol)

OD sample corrected = OD of sample with blank and background readings subtracted

OD spiked corrected = OD of spiked sample with blank and background readings subtracted

Analyte Spike = amount of Analyte spiked (pmol/nmol) into the sample well.

11. Typical Data

Data provided **for demonstration purposes only**.

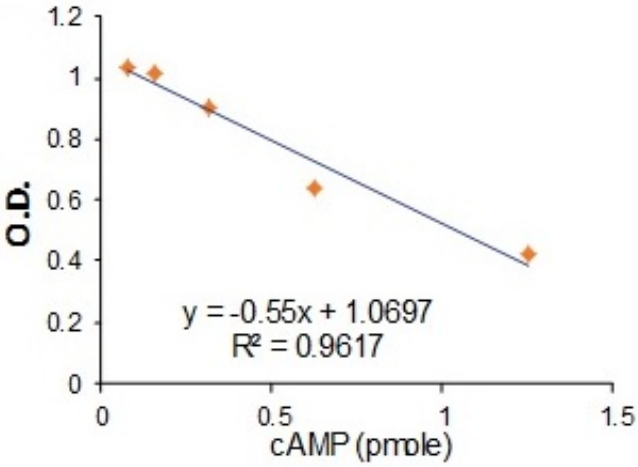


Figure 1. cAMP Standard Curve following the kit protocol.

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.co.jp/contactus (Japan)