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ab234585 Colorimetric ELISA cAMP Assay Kit

For the detection of cAMP activity in cells, tissue, urine, plasma or culture medium.

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

Table of Contents

1. Overview

Colorimetric ELISA cAMP Assay Kit (ab234585) is based on the competition between HRP-labeled cAMP and free cAMP for a fixed number of cAMP antibody binding sites. HRP-cAMP is displaced from the HRP-cAMP/anti-cAMP antibody complex by unlabeled free cAMP. In the absence of cAMP, HRP-cAMP conjugate is bound to anti-cAMP antibody exclusively. However, the unlabeled free cAMP in the test samples competes for anti-cAMP antibody with the HRP-cAMP antibody conjugate, therefore inhibits the binding of HRP-cAMP to anti-cAMP antibody. The kit provides the sensitive method for detecting adenylate cyclase activity in biochemical or cell-based assay system. Compared to other ELISA cAMP assay kits, our kit eliminates the tedious acetylation step, and provides the ready-to-use Anti-cAMP Ab coated 96-well plate and HRP substrate Green Probe to quantify the HRP activity. The color product formed is proportional to the activity of HRP-cAMP.

Prepare samples.

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Add cAMP standard or test samples into the anti-cAMP coated 96-well plate and incubate at room temperature for 5-10 minutes.

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Add 1X HRP-cAMP conjugate and Incubate at room temperature for 3 hours.

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Wash wells 4 times with Washing Buffer. Add Green Probe and incubate at room temperature for 1 to 3 hours

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Monitor absorbance increase at 405, 650 or 740 nm.

2. Materials Supplied and Storage

Store kit at 4°C in the dark 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.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity 1 plate	Quantity 10 plates	Storage temperature (before prep)	Storage temperature (after prep)
cAMP Standard	1 vial (33 µg)	1 vial (33 µg)	-20°C	-20°C
Assay Buffer	20 mL	1 x 100 mL	4°C	4°C
HRP-cAMP Conjugate	1 vial	1 vial	-20°C	-20°C
10X Wash Solution	10 mL	100 mL	4°C	4°C
Cell Lysis Buffer	10 mL	100 mL	4°C	4°C
Anti-cAMP Ab Coated 96-Well Plate	1	10	4°C	4°C
Green Probe	10 mL	100 mL	4°C	4°C

Δ Note: Do not freeze Anti-cAMP Ab Pre-coated 96-well plate. Store it at 4°C.

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 405, 650 or 740 nm.

4. 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.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 cAMP Standard

Prepare 100 µM cAMP stock solution by adding 1 mL of Assay Buffer to the vial of cAMP Standard. Unused reconstituted 100 µM cAMP stock solution should be aliquoted and stored at -20°C.

5.2 Assay Buffer

Ready to use as supplied.

5.3 HRP-cAMP Conjugate

1. Prepare 50X HRP-cAMP conjugate stock solution by adding 55 µL (for 1 plate kit) or 550 µL (for 10 plate kit) of Assay Buffer into the vial of HRP-cAMP Conjugate.
2. Make 1:50 dilution with Assay Buffer to have 1X HRP-cAMP conjugate working solution before use. Store it on ice or 4°C.
   Δ Note25 µL of 1X HRP-cAMP conjugate working solution is enough for one assay point; prepare appropriately volume for single use only.
   Δ Note: The unused 50X HRP-cAMP conjugate stock solution should be divided into single use aliquots and stored them at -20°C.

5.4 10X Wash Solution

Prepare 1X washing solution by adding 1 mL of 10X Wash Solution to 9 mL distilled water.

5.5 Cell Lysis Buffer

Ready to use as supplied.

5.6 Anti-cAMP Ab Coated 96-Well Plate

Ready to use as supplied.
Δ Note: Do not freeze Anti-cAMP Ab Pre-coated 96-well plate. Store it at 4°C.

5.7 Green Probe

Ready to use as supplied.

6. 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. Make 1:10, 1:100 and 1:3 serial dilutions of the 100 µM cAMP stock solution in Assay Buffer to have 10,000, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003 and 0 nM cAMP diluted solutions.
2. Store on ice or 4°C.

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Cell Samples:

1. For adherent cells: Plate cells overnight in growth medium at 30,000 -100,000 cells/well for a 96-well plate.
2. 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.
3. Treat cells as desired: The following is an example of Hela cells treated with Forskolin to induce cAMP in a 96-well plate format.
   a). Aspirate off cell growth medium, add 100 µL/well 100 µM Forskolin in Hanks and 20 mM Hepes buffer (HHBS), incubate in a 5% CO^2, 37°C incubator for 15 minutes;
   b). Aspirate off cell solution after the incubation, add 100 µL/well of Cell Lysis Buffer, and incubate at room temperature for another 10 minutes. This cell lysate can be assayed directly or diluted in Assay Buffer.
   Δ 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.

7.2 Tissue Samples:

1. It is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen) due to quick metabolism of cyclic nucleotides in tissue.
2. Weigh the frozen tissue and add 10-20 µL/mg of cell lysis buffer.
3. Homogenize the sample on ice. Spin at top speed for 5 minutes and collect the supernatant.
4. The supernatant may be assayed directly.

7.3 Urine, Plasma and Culture Medium Samples:

1. Urine and plasma may be tested directly with 1:200 to 1:1000 dilutions in 1X Lysis Buffer.
2. Culture medium can also be tested with 1:10 to 1:200 dilutions in Lysis Buffer.
   Δ Note: RPMI medium may contain > 350 fmol/µL cAMP.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 cAMP assay:

1. All the assay wells will be prepared in the following orders: A) cAMP standards, control, or tests samples; B) HRP-cAMP conjugate.
2. Add 75 µL/well of the cAMP diluted standard solution (from Step 6) and test samples into each well of the anti-cAMP Ab coated 96-well plate. Incubate at room temperature for 5 to 10 minutes.
3. Add 25 µL/well of 1X HRP-cAMP conjugate working solution (from Step 5.3). Incubate at room temperature for 3 hours by placing the plate on shaker.
4. Aspirate plate contents, and wash 4 times with 200 µL/well of 1X wash solution (from Step 5.4).
5. Add 100 µL/well of Green Probe into each well, and incubate at room temperature for 60 minutes to 3 hours, protected from light.
6. Monitor the absorbance increase at 405nm, 650 nm, or 740 nm using an absorbance plate reader.

9. FAQs / Troubleshooting

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

10. Typical Data

Data provided **for demonstration purposes only.**

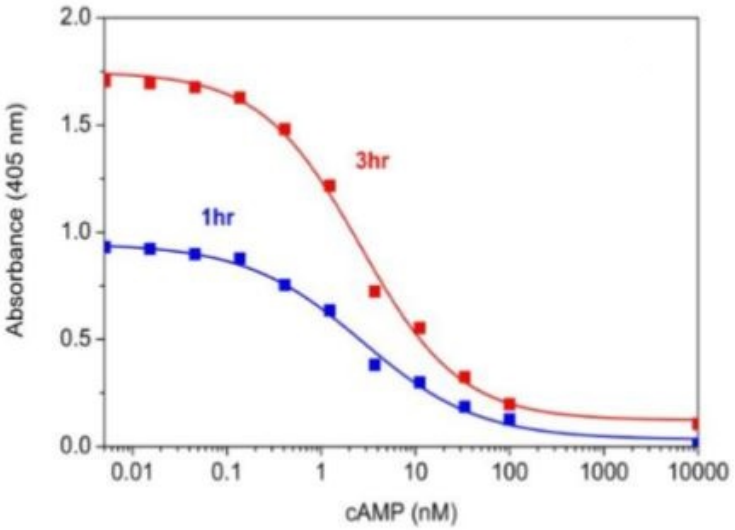

Figure 1. cAMP dose response was measured with Colorimetric ELISA cAMP Assay Kit (ab234585) in a clear 96-well plate with a microplate reader. The kit can detect as low as 0.1 nM cAMP in a 100 µL reaction volume at 405nm after incubation with Green Probe for 1 hour (blue line) and 3 hours (red line).

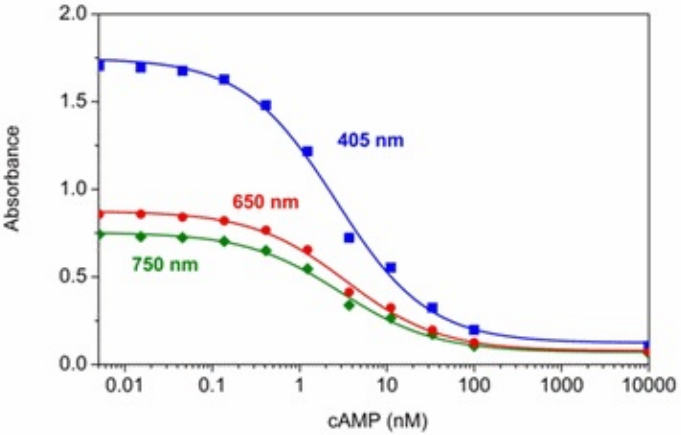

Figure 2. cAMP dose response was measured with Colorimetric ELISA cAMP Assay Kit (ab234585) in a clear 96-well plate with a microplate reader. The Absorbance can be read at 405 nm (blue line), 650 nm (red line) or 740 nm (Green line), the data are from the incubation with Green Probe for 3 hours.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

For all technical or commercial enquiries please go to:

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