Screen Quest™ Colorimetric ELISA cAMP Assay Kit

Catalog number: 36370, 36371
Unit size: 1 plate, 10 plates

	Component	Storage	Amount
		Cat No. 36370	Cat No. 36371
Component A: cAMP Standard	Refrigerated (2-8 °C), Minimize light exposure	1 vial (33 µg)	1 vial (33 µg)
Component B: Assay Buffer	Refrigerated (2-8 °C)	1 bottle (20 mL)	1 bottle (100 mL)
Component C: HRP-cAMP Conjugate	Refrigerated (2-8 °C), Minimize light exposure	1 vial	1 vial
Component D: 10X Wash Solution	Refrigerated (2-8 °C)	1 bottle (10 mL)	1 bottle (100 mL)
Component E: Cell Lysis Buffer	Refrigerated (2-8 °C)	1 bottle (10 mL)	1 bottle (100 mL)
Component F: Anti-cAMP Ab Coated 96-Well Plate	Refrigerated (2-8 °C), Minimize light exposure	1 plate	10 plates
Component G: Amplite™ Green	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (10 mL)	1 bottle (100 mL)

OVERVIEW

Adenosine 3', 5' cyclic monophosphate (cAMP) is an important second messenger in intracellular signal transduction. Monitoring levels of cAMP is one of the most common ways to screen for agonists and antagonists of GPCRs. Screen Quest™ Colorimetric ELISA cAMP Assay Kit is based on the competition between HRP-labeled cAMP and non-labeled cAMP. 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 sample competes for anti-cAMP antibody with the HRP-cAMP antibody conjugate, therefore inhibits the binding of HRP-cAMP to anti-cAMP antibody. Our Screen Quest™ Colorimetric ELISA cAMP Assay Kit provides a 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. The kit uses Amplite™ Green as a colorimetric substrate to quantify the HRP activity. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

AT A GLANCE

Protocol Summary

1. Prepare samples
2. Add 75 µL/well of cAMP standard or test samples into the anti-cAMP coated 96-well plate
3. Incubate at room temperature for 5-10 mins
4. Add 25 µL/well of 1X HRP-cAMP Conjugate
5. Incubate at room temperature for 3 hours
6. Wash 4 times with 200 µL/well Washing Buffer
7. Add 100 µL/well of Amplite™ Green
8. Incubate at room temperature for 1 to 3 hours
9. Monitor absorbance increase at 405, 650 or 740 nm

Important Do not freeze Anti-cAMP Ab Pre-coated 96-well plate (Component F), store it at 4°C. Allow all the kit components to warm to room temperature before using them. Some material might be stick to the vial cap during the shipment. Briefly centrifuge the vial to collect all the content.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	405, 650, or 740 nm
Recommended plate	Clear plate (Component F)

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. cAMP stock solution (100 µM)
Add 1 mL of Assay Buffer (Component B) to the vial of cAMP Standard (Component A).

2. HRP-cAMP conjugate stock solution (50X)
Add 55 µL (**Cat. # 36370**) or 550 µL (**Cat. # 36371**) of Assay Buffer (Component B) into the vial of HRP-cAMP Conjugate (Component C).

Note The unused 50X HRP-cAMP conjugate stock solution should be divided into single use aliquots and stored them at -20 ° C.

3. Washing solution (1X)
Add 1 mL of 10X Wash Solution (Component D) to 9 mL distilled water.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/36370

cAMP standard
Make 1:10, 1:100 and 1:3 serial dilutions of cAMP standards in Assay Buffer (Component B) to have 10,000, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0.003 nM cAMP diluted solutions. Store on ice or 4°C.

PREPARATION OF WORKING SOLUTION

HRP-cAMP Conjugate working solution
Make 1:50 dilution with Assay Buffer (Component B) to have 1X HRP-cAMP conjugate working solution before use. Store it on ice or 4°C.

Note 25 µL of 1X HRP-cAMP conjugate working solution is enough for one assay point; prepare appropriately volume for single use only.

SAMPLE EXPERIMENTAL PROTOCOL

Prepare samples

1. Treat cells as desired:The following is an example of Hela cells treated with Forskolin to induce cAMP in a 96-well plate format: 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. Aspirate off cell solution after the incubation, add 100 µL/well of Cell Lysis Buffer (Component E), and incubate at room temperature for another 10 minutes. This cell lysate

can be assayed directly or after diluted in Assay Buffer (Component B).

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.

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

3. Urine, Plasma and Culture Medium Samples:Urine and plasma may be tested directly with 1:200 to 1:1000 dilutions in 1X 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.

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 and test samples into each well of the anti-cAMP Ab coated 96-well plate (Component F). We recommended duplicating the assays for each standard and testing sample. Incubate at room temperature for 5 to 10 minutes.

3. Add 25 µL/well of 1X HRP-cAMP Conjugate working solution. 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.

5. Add 100 µL/well of Amplite™ Green (Component G) into each well, and incubate at room temperature for 60 mins to 3 hours, protected from light.

6. Monitor the absorbance increase at 405 nm, 650 nm, or 740 nm using an absorbance plate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate cAMP samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

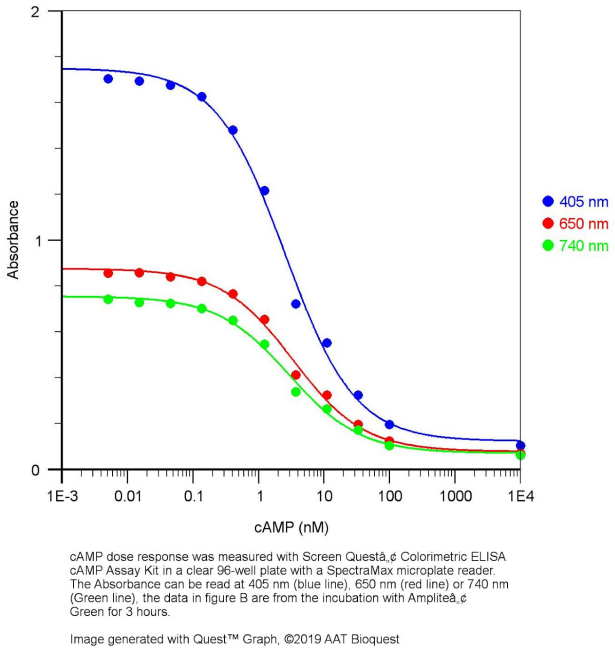

Figure 1. cAMP dose response was measured with Screen Quest™ Colorimetric ELISA cAMP Assay Kit in a clear 96-well plate with a SpectraMax microplate reader. The Absorbance can be read at 405 nm (blue line), 650 nm (red line) or 740 nm (Green line), the data in figure B are from the incubation with Amplite™ Green for 3 hours.

DISCLAIMER

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