Screen Quest™ Fluorimetric ELISA cAMP Assay Kit

Catalog number: 36373, 36374
Unit size: 1 plate, 10 plates

Component	Storage	Amount (Cat No. 36373)	Amount (Cat No. 36374)
Component A: cAMP Standard	Freeze (< -15 °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	Freeze (< -15 °C), Minimize light exposure	1 vial	5 vials
Component D: 10X Wash Solution	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (10 mL)	1 bottle (100 mL)
Component E: Cell Lysis Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)	1 bottle (100 mL)
Component F: 3% H2O2	Refrigerated (2-8 °C), Minimize light exposure	1 vial (50 µL)	1 vial (250 µL)
Component G: Amplite™ Red	Freeze (< -15 °C), Minimize light exposure	1 vial	1 vial
Component H: Anti-cAMP Ab Coated 96-Well Plate	Refrigerated (2-8 °C), Minimize light exposure	1 plate	10 plates
Component I: Substrate Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (10 mL)	1 bottle (100 mL)

OVERVIEW

Screen Quest™ Fluorescence ELISA cAMP Assay Kit provides an optimized assay method for monitoring the activation of adenylyl cyclase in G-protein coupled receptor systems. The assay is based on the competition for a fixed number of antibody binding sites 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™ Fluorometric cAMP Assay Kit provides a sensitive method for detecting adenylate cyclase activity. Compared to other commercial ELISA cAMP assay kits, this cAMP assay kit only requires a single wash step to remove unbound material prior to the development step. It also eliminates the tedious acetylation step. The kit uses Amplite® Red as a fluorogenic HRP substrate to quantify the HRP activity. The fluorescent product formed is proportional to the activity of HRP-cAMP conjugate.

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 2 hours
6. Wash 4 times with 200 µL/well Washing Buffer
7. Add 100 µL/well of Amplite™ Red
8. Incubate at room temperature for 15 to 60 minutes
9. Monitor fluorescence increase at Ex/Em = 540/590 nm

Important Note

Do not freeze Anti-cAMP Ab Pre-coated 96-well plate (Component H), 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

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 nm
Recommended plate	Solid black (Component H)

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

cAMP stock solution (100 µM)

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

Note: The unused cAMP stock solution (100 µM) should be aliquoted and stored at -20 °C.

HRP-cAMP conjugate stock solution (50X)

Add 55 µL (**Cat. # 36373**) or 110 µL (**Cat. # 36374**) 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.

Washing solution (1X)

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

Amplite™ Red stock solution (200X)

Add 50 µL (**Cat. # 36373**) or 500 µL (**Cat. # 36374**) of DMSO to the vial of Amplite™ Red (Component G).

Note: 0.5 µL of 200X Amplite™ Red stock solution is enough for one assay point. The unused reconstituted stock solution should be aliquoted and stored at -20 °C

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/36373

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.

Amplite™ Red working solution

Add 50 µL of Amplite™ Red stock solution (200X) and 11.5 µL of 3% H_2O_2 (Component F) into 10 mL of Substrate Buffer (Component I).

Note: The Amplite™ Red working solution is not stable, use it promptly.

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 H). 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 2 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™ Red working solution into each well, and incubate at room temperature for 10 mins to 2 hours, protected from light.
6. Monitor the fluorescence increase at Ex/Em = 540/590 nm (cutoff 570 nm) by using a fluorescence plate reader (top read mode).

EXAMPLE DATA ANALYSIS AND FIGURES

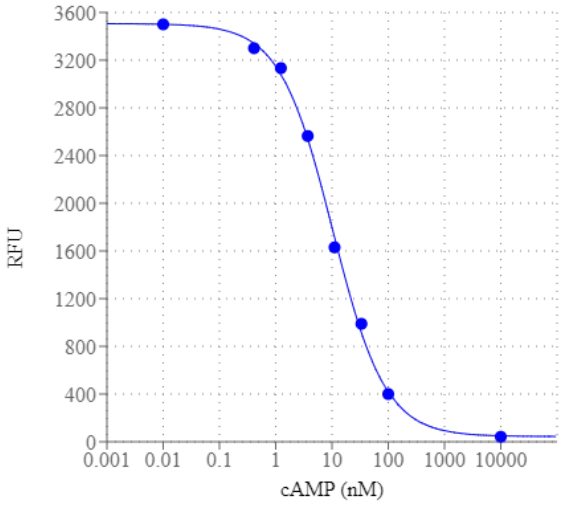

Figure 1. cAMP dose response was measured with Screen Quest™ Fluorimetric ELISA cAMP Assay Kit in a solid black 96- well plate with a Gemini microplate reader.

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