Signosis
Innovative Plate Assay Solutions

cAMP ELISA Kit

Catalog Number EA-7060 **(For Research Use Only)**

Introduction

Cyclic adenosine monophosphate (cAMP) is a small molecule second messenger that plays a key role in many biological processes, especially cell signaling downstream of GPCRs. cAMP has many biological functions including the regulation of gene expression, neuron signaling, cardiac function, and hormone release. Measuring cAMP levels in cells is important because it can help researchers study how drug compounds affect GPCR activity and signaling pathways inside the cell. Signosis has developed a competition-binding ELISA kit that can analyze cAMP in biological samples.

Principle of the assay

Each well of the plate is coated with anti-cAMP antibody, which binds to both cAMP and the cAMP tracer provided in this kit. The cAMP tracer is held at a constant concentration while the concentrations of cAMP in the samples will vary. As a result, the amount of cAMP tracer that binds to the antibody is inversely proportional to the concentration of cAMP in the well. After washing the wells remove any unbound components, DTNB is added to the wells to detect the cAMP tracer that remains bound to the immobilized antibody in the well. DTNB reacts with the cAMP tracer to form a yellow product that can be measured spectrophotometrically at an absorbance of 412 nm.

Materials Provided

- 96-well plate coated with anti-cAMP antibody (4°C)
- cAMP Tracer (-80°C)
- cAMP Standard (-20°C)
- 1x Diluent Buffer (4°C)
- 5x Assay Wash Buffer (4°C)
- DTNB Detection Reagent (-20°C)

Reagent preparation for experiment

- Dilute the 5x Assay wash buffer to 1x Assay wash buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O .

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use lysis buffer. Cell Lysis Buffer for ELISA available (Catalog# EA-0001). Follow protocol in Cell Lysis Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X Diluent buffer, for example, add 80ul sample in 720ul 1X Diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Assay procedure

1. Standard curve preparation: First, take eight new tubes and label them 1-8. In tube 1, add 120 µL of the provided 1mM cAMP standard. Then, for tubes 2-8, add 60 µL of PBS to each tube. Transfer 60 µL of the 1mM cAMP standard from tube 1 to the PBS in tube 2 and mix to make a 500µM cAMP standard. Then, transfer 60 µL of the 500µM cAMP standard from tube 2 to the PBS in tube 3 and mix to make an 250µM cAMP standard. Continue the serial dilutions until tube 7 is done. Leave tube 8 untouched as the PBS negative control.

Standard#	cAMP Concentration (µM)
1	1000
2	500
3	250
4	125
5	62.5
6	31.25
7	15.625
8	0

2. Add 50 µL of sample or standard to each well.
3. Prepare cAMP Binding mix by diluting cAMP Tracer 1:200 with 1x Diluent buffer
4. Add 50 µL of the cAMP Binding mix to each well.
5. Cover the plate with the provided plate seal and incubate at 4°C for 2 hours.
6. Aspirate each well and wash by adding 200 µL of 1x Assay wash buffer. Repeat the process three times for a total of three washes. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
7. Prepare cAMP Detection solution by diluting DTNB Detection reagent 1:20 with 1x Diluent buffer
8. Add 100 µL of the cAMP Detection solution to each well.
9. Cover the plate and incubate at 37°C for 10 minutes.
10. Measure the absorbance of the plate at 412 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader at Ex/Em: 340/460 nm.