

AB308320 Catlog – cAMP Assay Kit (Fluorometric)

A fluorometric method to measure cAMP levels in various sample types

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [abcam website](#)

Storage and Stability

Store kit at -20°C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
cAMP Assay Buffer	25 ml	-
Neutralizing Buffer	7 ml	-
cAMP Converter	200 µl	-20°C
cAMP Developer	1 vial	-20°C
cAMP Enzyme	200 µl	-20°C
cAMP Probe (in DMSO) (Avoid light)	1 vial	-20°C
cAMP Standard	1 vial	-20°C

Materials Required, Not Supplied

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

- 96-well black plate with flat bottom
- Multi-well fluorescence microplate reader
- 0.1 N HCl

Reagent Preparation

cAMP Assay Buffer: Warm both the buffers to room temperature (RT) before use.

Neutralizing Buffer: Warm both the buffers to room temperature (RT) before use.

cAMP Converter: Store at -20 °C. Thaw and keep on ice while using.

cAMP Developer: Reconstitute each of the vials in 220 µl cAMP Assay Buffer. Pipette up and down to dissolve completely. Divide into aliquots and store at -20 °C. Keep on ice while in use. Use within 2 months.

cAMP Substrate Mix: Dissolve the vial in 220 µl dH₂O. Pipette up and down to dissolve completely. Divide into aliquots and store at -20 °C, protected from light. Avoid repeated freeze/thaw cycles and use within two months. Stable for 2 months at -20 °C.

cAMP Enzyme: Reconstitute each of the vials in 220 µl cAMP Assay Buffer. Pipette up and down to dissolve completely. Divide into aliquots and store at -20 °C. Keep on ice while in use. Use within 2 months.

cAMP Probe (in DMSO): Warm to RT before use. Store at -20 °C.

cAMP Standard (10 mM): Reconstitute the vial in 200 µl dH₂O to make stock 10 mM cAMP Standard. Keep on ice while in use. Divide into aliquots and store at -20 °C. Use within 2 months. to dissolve completely. Divide into aliquots and store at -20 °C. Keep on ice while in use. Use within 2 months.

cAMP Assay Kit (Fluorometric) Protocol:

Sample Preparation: For Plant Tissue Samples:

1. Tissue samples (~10 mg) or cells pellets (~1 x 10⁷ suspension cells) should be rapidly homogenized in 500 µl 0.1 N HCl (not provided) and put on ice for 20 min. Centrifuge at 10,000 x g and 4 °C for 10 min and collect the supernatant.
2. For adherent cells, add 1 ml of 0.1 N HCl for every 35 cm² of surface area and incubate at 4 °C for 20 min. Scrape the cells off the surface with a cell scraper. Transfer to a centrifuge tube and spin down at 10,000 X g for 10 min and collect the supernatant.
3. Neutralize the cell or tissue lysate by using the Neutralizing Buffer at 2:1 ratio. For example, for 1 ml of lysate, add 0.5 ml of Neutralization Buffer to bring the pH around 7.

Δ Note: *If the Samples are not clear after neutralization, spin at 10,000 x g for 2 min to remove any insoluble components. Use the supernatant for the assay.*

4. Mix well and use 1-40 µl of each Sample into three replicate wells of a 96 well black plate labelled as Sample, Sample Background and Spiked Sample respectively.

Δ Note:

1. *Esterases in Samples may degrade cAMP quickly. Therefore, prepare Samples in 0.1N HCl to inactivate the esterases. For further testing, lysates can be stored at -70 °C for less than week.*
2. *For Unknown Samples, we suggest testing several doses to ensure that the readings do not exceed the signal from the Internal Standard (see below).*

Standard Curve Preparation:

Dilute stock 10 mM cAMP Standard to 1 mM (1 nmol/µl) cAMP Standard by adding 10 µl of stock 10 mM cAMP Standard to 90 µl cAMP Assay Buffer and mix well to generate 1 mM cAMP. Add 100 µl of 1 mM cAMP Standard to 900 µl cAMP Assay Buffer to generate 0.1 mM (100 pmol/µl) cAMP Standard.

Internal Standard Preparation:

Add 4 µl of 0.1 mM or 100 pmol/µl cAMP Standard into the Spiked Sample well. The Spiked Sample well is used as an Internal Standard to correct for any Sample interference. Adjust the final volume of all three wells to 50 µl with cAMP Assay Buffer.

Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
cAMP Assay Buffer	41 µl	43 µl
cAMP Converter	2 µl	2 µl
cAMP Developer	2 µl	2 µl
cAMP Substrate	2 µl	2 µl
cAMP Enzyme	2 µl	---
cAMP Probe	1 µl	1 µl

Add 50 µl of Reaction Mix to Sample and Spiked Sample wells and mix well.

Note: For Samples having background, add 50 µl of Background Control mix to the Sample Background well and use this for Sample correction.

Measurement:

Incubate the plate in the dark for 30 minutes at 37°C. Measure fluorescence (Ex/Em = 535/587 nm).

Calculation:

Subtract the Sample Background reading from its paired Sample reading to get the Sample Corrected reading. Correct for any Sample interference by subtracting the Sample RFU reading from the Spiked Sample RFU reading. Determine the cAMP amount (X) in the Sample wells based on the following equation:

$$\text{cAMP amount (pmol)} = (\text{RFU}_{\text{sample corrected}}) / [(\text{RFU}_{\text{spiked sample}}) - (\text{RFU}_{\text{sample}})] * 400$$

The cAMP concentration in the Sample is calculated as

$$C = X/V \times D = \text{pmol}/\mu\text{l} = \mu\text{mol}/\text{l} \text{ or } \mu\text{M}$$

Where:

X = Amount of cAMP from the calculation above (pmol)

V = Sample volume added per well (µl)

D = Sample dilution factor

400 = Amount spiked in Sample well (400 pmol)

cAMP MW = 329.21 g/mol.

Sample cAMP concentration can also be expressed in pmol/µg or nmol/mg of Sample.

Technical Support

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