Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

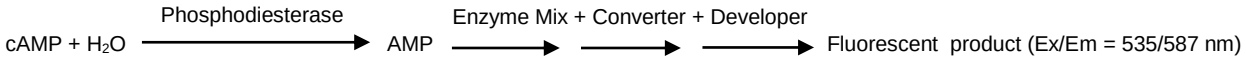
cAMP Assay Kit (Fluorometric)

05/21

(Catalog # K2087-100; 100 assays; Store at -20 °C)

I. Introduction:

Cyclic Adenosine Monophosphate (cyclic AMP or cAMP) is a vital second messenger found in eukaryotic and prokaryotic cells. It regulates many biological pathways such as glucose and lipid metabolism, cell growth and differentiation, gene transcription, protein expression etc. Additionally, deregulation of cAMP signaling has been shown in cancer development and progression. The main effectors of cAMP include protein kinase A (PKA), guanine-nucleotide-exchange factor and cyclic nucleotide gated ion channels. PKA is a tetrameric holoenzyme consisting of two catalytic subunits and a regulatory subunit dimer and its activity is dependent on the cellular levels of cAMP. Upon elevation of intracellular cAMP levels, cAMP binds to the regulatory subunits of PKA thereby causing a conformational change and releasing the catalytic subunit, which can then phosphorylate the substrate proteins and increase the transcription of target genes. **BioVision's cAMP Assay Kit** provides a rapid, sensitive, fluorometric method to measure cAMP levels in various sample types. In this assay, cAMP is converted to AMP in the presence of a phosphodiesterase. AMP then goes through several enzymatic reactions to produce an intermediate, which reacts with a probe to generate a fluorescent signal measured at Ex/Em = 535/587 nm. The fluorescent signal is proportional to the amount of cAMP present in the sample. The assay is simple, easy to use, quick, sensitive, and is high-through adaptable. It can measure lower than 0.2 µM cAMP levels in various samples.



II. Applications:

- To measure cAMP levels in various cell and tissue lysates
- To study the regulation of AMPK by cAMP
- To study key cellular processes such as Ca^{2+} signaling, glucose uptake, lipid uptakes and oxidation

III. Sample Types:

- Adherent or suspension cells i.e. HeLa, Jurkat cells.
- Animal Tissues i.e. liver, kidney, lung etc.

IV. Kit Contents:

Components	K2087-100	Cap Code	Part Number
cAMP Assay Buffer	25 ml	WM	K2087-100-1
Neutralizing Buffer	7 ml	NM	K2087-100-2
cAMP Converter	200 µl	Blue	K2087-100-3
cAMP Developer	1 vial	Green	K2087-100-4
cAMP Substrate Mix	1 vial	Amber	K2087-100-5
cAMP Enzyme	1 vial	Purple	K2087-100-6
cAMP Probe (in DMSO)	200 µl	Red	K2087-100-7
cAMP Standard	1 vial	Orange	K2087-100-8

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **cAMP Assay Buffer and Neutralization 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** and **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 Substrate Mix:** Dissolve the vial in 220 µl dH_2O. 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 Probe (in DMSO):** Warm to RT before use. Store at -20 °C.
- **cAMP Standard (10 mM):** Reconstitute the vial in 200 µl dH_2O 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.

VIII. cAMP Assay Protocol:

1. Sample Preparation:

A. Tissue samples (~10 mg) or cells pellets (~1 x 10^7 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.

B. For adherent cells, add 1 ml of 0.1 N HCl for every 35 cm^2 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.
C. 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. **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.
D. Mix well and use 1-40 µl of each Sample into three replicate wells of a 96 well black plate labeled as **Sample, Sample Background** and **Spiked Sample** respectively.

Notes:

- 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.
- For Unknown Samples, we suggest testing several doses to ensure that the readings do not exceed the signal from the Internal Standard (see below).

2. **Standard 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.
3. **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.
4. **Reaction Mix Preparation:** 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.

5. **Measurement:** Incubate the plate for 30 min at 37 °C and measure the fluorescence at Ex/Em = 535/587 nm.
6. **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:

$$\textbf{cAMP amount (pmol)} = \left(\frac{(\mathrm{RFU}_{\text{Sample (corrected)}})}{(\mathrm{RFU}_{\text{(Spiked Sample)}}) - (\mathrm{RFU}_{\text{Sample}})}\right) * 400$$

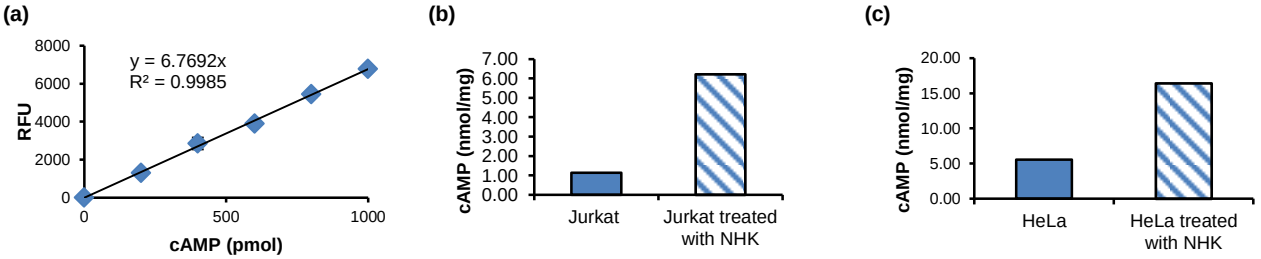
The cAMP concentration in the Sample is calculated as

C = X/V x D = pmol/µl = µmol/l or µM

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.



Figures: (a) cAMP Standard Curve. (b) Measurement of cAMP in Jurkat cells without or with 100 µM NHK477 treatment. (c) Measurement of cAMP in HeLa cells without or with 100 µM NHK477 treatment. Assays were performed following the protocol.

IX. Related Products:

AMP Colorimetric Assay (K229)
cAMP Direct Immunoassay Kit (Colorimetric) (K371)
ADP Colorimetric/Fluorometric Assay Kit (K355)
Adenosine Assay Kit (Fluorometric) (K327)
Total Phosphodiesterase Activity Kit (Fluorometric) (K927)
cAMP Phosphodiesterase Activity Assay Kit (K2013)

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