Cyclic AMP (cAMP) Colorimetric Assay **Catalog Number: TBS2073**

For the quantitative determination of cAMP concentrations in cell culture supernatants, tissue, and serum.

INTRODUCTION

Cyclic AMP (cAMP, Adenosine 3',5'-cyclic monophosphate) is one of the most important second messengers involved as a modulator of physiological processes. A few hormones are known to activate cAMP through the action of the enzyme Adenylate cyclase which converts ATP to cAMP. cAMP has been shown to be involved in the cardiovascular and nervous systems, immune mechanisms, cell growth and differentiation, and general metabolism.

PRINCIPLE OF THE ASSAY

This assay kit is a competition enzyme-linked immunoassay designed to measure cAMP levels in cells or tissues. An anti-mouse IgG was pre-coated onto a microplate. The cAMP in the test sample competes with cAMP-HRP for binding to an anti-cAMP mouse monoclonal antibody. Following the incubation and wash step, an ultra-sensitive substrate TMB is added for color development. Because of the competitive nature of this assay, the color intensity is inversely proportional to the quantity of sample cAMP levels. Measurement of absorbance using the cAMP Standard allows calculating the absolute amount of cAMP in a sample. This assay provides a flexible, accurate, simple, and time-saving approach for cAMP detection.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Precoated Microplate	TBS2073A	96 well polystyrene microplate (12 strips of 8 wells) coated with anti-mouse IgG antibody.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
cAMP Standard	TBS2073B	50 µL of cAMP (1 mM).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS2073C	110 µL of cAMP-HRP (25x).	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS2073D	1.1 mL of anti-cAMP antibody (5x).	
Assay Diluent	TBS2073E	20 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x)	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use. cAMP can precipitate when frozen, incubate the vial in the water bath (up to 50°C) with occasional mixing until the precipitate is dissolved.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

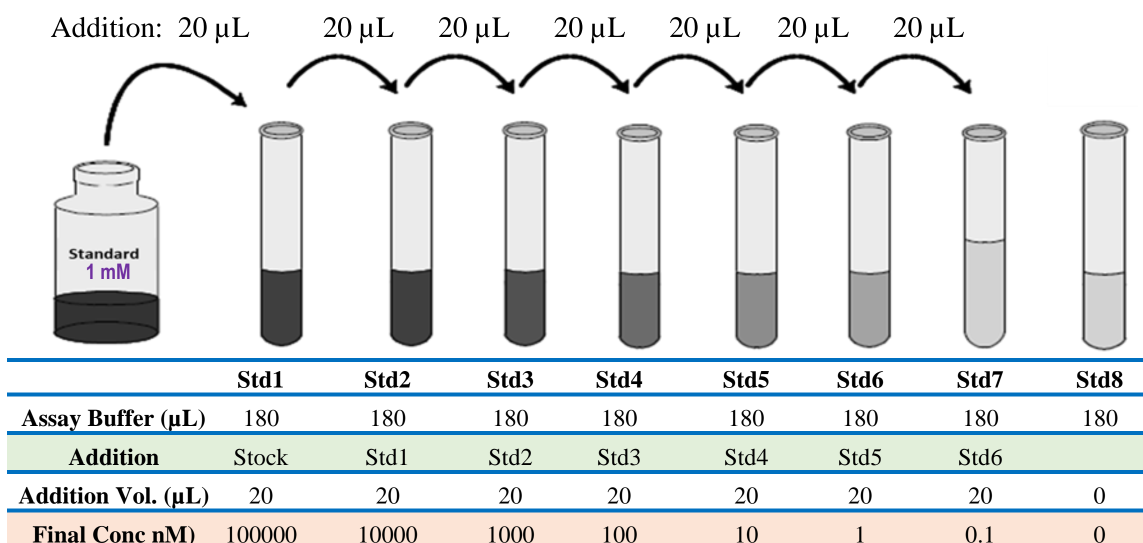
Detection A working solution: Dilute 1 mL of Detection A stock into 4.0 mL Assay Buffer.

Detection B working solution: Dilute 100 µL of Detection B stock into 2.4 mL Assay Buffer.

cAMP Standard Preparation: Label test tubes as #1 through #8. Pipet 180 µL of 1x Assay Diluent into tubes #1 to #8 as diagram below.

1. Add 20 µL of the cAMP Standard stock solution (1 mM) to tube #1 and mix.
2. Make 10x serial dilutions of the standard using Tube #1(100 µM standard solution) from Tube #2 through #7 with sequential transfer of 20 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube #1 through #7 will be 100000, 10000, 1000, 100, 10, 1, and 0.1nM. Tube# 8 is 0 nM.

Fig.1 Diagram for cAMP standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	180	180	180	180	180	180	180	180
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	20	20	20	20	20	20	20	0
Final Conc nM)	100000	10000	1000	100	10	1	0.1	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 50 µL of cAMP standard, or sample, or control to each well. Add 100 µL of 1x Assay Diluent to the blank well.
2. Add 25 µL of cAMP-HRP to the above standard, sample and blank of each well, thoroughly mix.
3. Add 50 µL of Detection solution to each well, except for the blank well.
4. Cover the plate and incubate at **RT for 2 hours with gentle shaking.**
5. Discard the liquid and blot the plate against clean paper towels.
6. Wash 3 times with 200 µL Wash Buffer (*Complete removal of the liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20 minutes with shaking** (*Protect from light*). The color becomes blue**.**
8. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
9. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 542 nm or 570 nm. If wavelength correction is not available, subtract readings at 542 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the human concentrations versus the log of the O.D. and the best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 2 is an example of typical Data.

SENSITIVITY

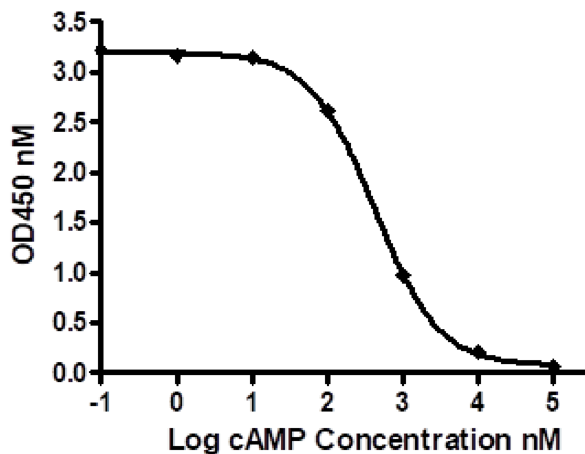
The minimum detectable dose (MOD) of cAMP is typically 1 nM.

The Intra-assay CV and the Inter-assay CV are <10%.

SPECIFICITY

This assay recognizes cAMP.
No cross-reactivity with others.

Fig2. cAMP Standard Curve



RELATIVE PRODUCTS

cAMP ELISA Fluorometric Assay (TBS2081)
cGMP ELISA Colorimetric Assay (TBS2074)
Human p-Tau-217 ELISA (TBS3293)
Human p-Tau-181 ELISA (TBS3294)
Human Total Tau ELISA (TBS3295)
Human p-Tau-231 ELISA (TBS3296)
Human AD7c NTP (TBS3297)
Human Amyloid ß40 ELISA (TBS3298)
Human NF-L ELISA (TBS32101)
Human Total Amyloid ß ELISA (TBS32104)
Human UCHL1/PGP9.5 ELISA (TBS32107)
Human Gamma H2AX ELISA (TBS3265)
Human H2AX ELISA (TBS3266)
Human IL-4 ELISA (TBS3221)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IL-33 ELISA (TBS4245)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)

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