Version 16-09/24

User's Manual

cAMP ELISA Kit

REF **DEIA-CA02**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit uses a competitive ELISA method to measure cAMP levels in cell extracts or in vitro adenylate cyclase assays.

General Description

Cyclic adenosine monophosphate (cAMP) is an intracellular second messenger that is derived from the conversion of adenosine triphosphate catalysed by adenylyl cyclase (AC). Protein kinase A (PKA), the main effector of cAMP, is a dimeric protein kinase consisting of two catalytic subunits and two regulatory subunits. When cAMP binds to the regulatory subunits of PKA, it leads to the dissociation and activation of PKA, which allows the catalytic subunit of PKA to phosphorylate target proteins, thereby regulating various physiological functions and metabolic processes in cellular function. cAMP's notable characteristic lies in its ability to tightly regulate a wide range of physiological processes, including metabolism, homeostasis, secretion, muscle contraction, cell proliferation and migration, immune response, and gene transcription. The intracellular cAMP levels are regulated by cyclic nucleotide phosphodiesterases (PDEs). It is recognized that the action of phosphodiesterases is essential for the spatiotemporal regulation of cAMP levels. Research suggests that inhibitors of cAMP-specific phosphodiesterases can be used to treat human diseases.

People have been looking for a method to quickly, sensitively, specifically and reproducibly detect the content of cAMP. This can help people quickly understand the regulatory role of cAMP in various physiological processes. And it will be applied to related drug development and drug screening.

Principles of Testing

This kit uses a competitive ELISA method to measure the level of cAMP in cell extracts or in vitro adenylate cyclase assays. Antibodies are coated on ELISA plates. cAMP in the sample competes with a fixed amount of HRP Conjugate cAMP for binding to anti-cAMP monoclonal antibodies. The cAMP standards with known concentration are used to make a standard curve. The OD value at a wavelength of 450nm is measured. The absorbance OD value is inversely proportional to the concentration of cAMP in the sample. The concentration of cAMP in the sample can be calculated based on the curve obtained with the cAMP standards and the measured OD value. The IC50 (50% B/B0) of this kit is about 12pmol/mL, and the detection limit is about 0.15 pmol/mL.

Reagents And Materials Provided

1. cAMP Standard, 100 µl, -20°C ~ -80°C
2. cAMP-HRP Conjugate (1000×), 20 µl, -20°C ~ -80°C
3. cAMP monoclonal antibody (1000×), 20 µl, -20°C ~ -80°C
4. Assay Buffer (10×), 30 ml, 2~8°C
5. Neutralizing Reagent, 8 ml, 2~8°C
6. Sample Diluent, 30 ml, 2~8°C

7. TMB Substrate, 18 ml, 2~8°C
8. Stop Solution, 8 ml, 2~8°C
9. Goat anti-Mouse IgG Coated plate, 8 wells × 12 strips, 2~8°C

Materials Required But Not Supplied

1. ELISA reader (detection wavelength 450 nm, reference wavelength 630 nm)
2. Pipette and disposable tip
3. EP tube
4. Microplate oscillator
5. Absorbent paper
6. Deionized water

Storage

cAMP Standard, cAMP-HRP Conjugate (1000×), cAMP monoclonal antibody (1000×): -20°C~-80°C

Other Reagents: 2-8°C

Specimen Collection And Preparation

1. Tissue samples: Freshly collected tissue samples must be immediately frozen in liquid nitrogen. Remove the frozen sample, weigh it, and add 5-10 times the volume of 0.1M HCl. Homogenize it on ice with a homogenizer. Then centrifuge it at room temperature for 5 minutes (>600 g). Keep the supernatant and use it directly for experiments or dilute it with 0.1M HCl.

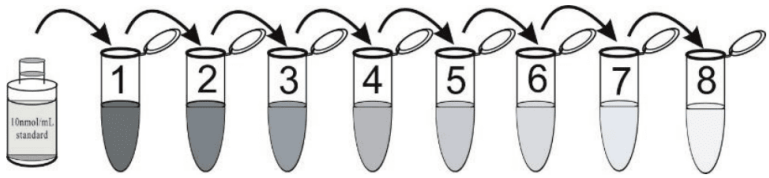
2. Cell samples: Remove the culture medium and add an appropriate amount of 0.1M HCl (adding 0.1%~1% Triton X-100 to 0.1M HCl can enhance the cell lysis effect). Leave it for 10 minutes and observe during this period to confirm whether the cells are lysed. If the lysis is not sufficient, you can add another 10 minutes until the cells are completely lysed. Centrifuge it at room temperature for 5 minutes (600 g). Keep the supernatant for detection by this kit. **Note: The above concentration of Triton X-100 will not affect the binding of the sample to the plate, but it may increase the background value. It is recommended to dilute the standards with Triton X-100 containing the same concentration to eliminate errors.**

3. Urine, plasma and culture medium: Add 10µl concentrated hydrochloric acid (12M) to every 1ml sample and mix well. Centrifuge at room temperature for 5min (600g) and save the supernatant for detection. Plasma, serum, whole blood and tissue homogenates usually contain phosphodiesterases and large amounts of immunoglobulins (Ig), which will interfere with the experiment. Treating the sample with 0.1M HCl can inactivate the phosphodiesterase and reduce the concentration of immunoglobulins, making it suitable for use in this kit. Phosphodiesterase and immunoglobulins can also be precipitated by adding 2% TCA (trichloroacetic acid) or removed by ultrafiltration centrifuge tubes with a molecular weight cutoff of 10 KD.

Reagent Preparation

1. Preparation of cAMP standard solution: Take 8 new EP tubes and label them 1-8#. Add 980 µl 0.1M

HCl to tube 1# and 500 µl 0.1M HCl to tubes 2-8# respectively. Add 20µl standard (10000 pmol/mL) to tube 1# and shake vigorously. Take out 500µl from tube 1# and add to tube 2# and shake vigorously. Use the same method to dilute tubes 3-8# (the cAMP concentrations of tubes 1-8# are 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 pmol/ml respectively). It is recommended to use the diluted standard within 15 minutes. In addition, label a new EP tube as B_0 (maximum binding, 0 pmol/ml) and add 500 µl of 0.1 M HCl.



2. Preparation of "1×Assay Buffer": Add the Assay Buffer (10×) (30 ml) of this kit into 270 ml of deionized water. **Note:** The prepared 1×Assay Buffer can be stored at room temperature for 3 months (but not beyond the shelf life of the kit)

3. Preparation of "1×cAMP-HRP Conjugate": dilute cAMP-HRP Conjugate (1000×) to 1× with the prepared 1× Assay Buffer at 1:1000. This working solution is further diluted 20 times as the HRP conjugate working solution for TA wells.

4. Preparation of "1× Anti-cAMP monoclonal antibody": Dilute Anti-cAMP monoclonal antibody (1000×) 1:1000 to the working solution using the prepared 1× Assay Buffer.

Notes:

1. All relevant reagents in this kit are pre-equilibrated to room temperature (20-25°C).
2. Add standards and samples to each well.
3. Add each reagent along the wall of the well to avoid cross contamination.
4. This kit provides detachable well strips, and users can choose the amount to use according to the number of samples. Please return unused wells to the original sealed bag and store at 4°C. Please place the well plate on the well plate rack provided with this kit.
5. Before adding the substrate, make sure there is no residual liquid in the well.
6. The stop solution is corrosive, please use it with caution.

Assay Procedure

1. Add 50 µl Neutralizing Buffer to each well. TA (Total Activity) and blank wells are excluded.
2. Add 50 µl 0.1M HCl to NSB wells (Non-Specific Binding) and B_0 wells.
3. Take 50 µl of each concentration standard solution to the corresponding wells. .
4. Take 50 µl of sample solution to the corresponding wells.
5. Take 50 µl of 1× Assay Buffer working solution to each NSB well.
6. Take 50 µl of 1× cAMP-HRP Conjugate working solution to each well (not added to TA and blank wells).
7. Take 50 µl of 1× Anti-cAMP monoclonal antibody to each well (not added to TA, blank and NSB wells).
8. Incubate at room temperature for 2 h.
9. Empty and wash with Assay Buffer 4 times, pouring out the residual liquid each time.
10. Add 5 µl cAMP-HRP Conjugate working solution to the TA wells.

11. Add 150 µl TMB Substrate to each well and let stand at room temperature for about 10 minutes.
12. Add 50 µl Stop Solution to each well and immediately read on the microplate reader (detection wavelength is 450nm, reference wavelength is 630nm). Subtract the optical density value of the blank well from each reading.

Well	Neutralizing Buffer	0.1M HCl	Standard /Sample	cAMP-HRP Conjugate	Anti-cAMP monoclonal antibody	Assay Buffer
Blank	-	-	-	-	-	-
TA	-	-	-	5µl	-	-
NSB	50µl	50µl	-	50µl	-	50µl
B0	50µl	50µl	-	50µl	50µl	-
Standard /Sample	50µl	-	50µl	50µl	50µl	-

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S1	S1	SP1	SP16	SP17	SP32	SP33	SP48	SP49	SP64	SP65
B	Blank	S2	S2	SP2	SP15	SP18	SP31	SP34	SP47	SP50	SP63	SP66
C	NSB	S3	S3	SP3	SP14	SP19	SP30	SP35	SP46	SP51	SP62	SP67
D	NSB	S4	S4	SP4	SP13	SP20	SP29	SP36	SP45	SP52	SP61	SP68
E	B0	S5	S5	SP5	SP12	SP21	SP28	SP37	SP44	SP53	SP60	SP69
F	B0	S6	S6	SP6	SP11	SP22	SP27	SP38	SP43	SP54	SP59	SP70
G	B0	S7	S7	SP7	SP10	SP23	SP26	SP39	SP42	SP55	SP58	SP71
H	TA	S8	S8	SP8	SP9	SP24	SP25	SP40	SP41	SP56	SP57	SP72

S1-S8: Standards 1-8

SP1-SP72: samples

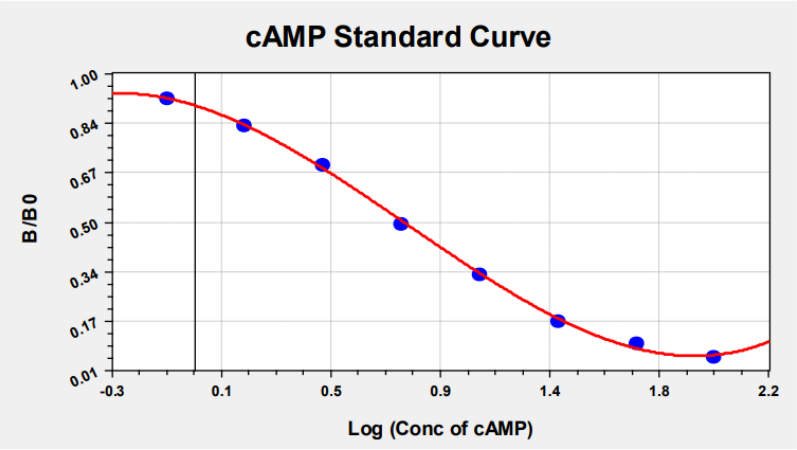
Calculation

1. Calculation of the net OD average of samples and standards:

   Net OD average = OD average - NSB well OD average
2. Calculation of binding rate (the percentage of binding rate of each concentration of standard to the maximum binding rate (B_0 well)):

   Binding rate (B/B_0) = (Net OD average / B0 well OD average) × 100
3. Use software to create a standard curve of binding rate (or net OD average) versus Log (cGMP standard concentration). Use three order polynomial linear regression curve fitting or exponential logistic curve fitting.
4. The cAMP concentration in the sample can be calculated by the binding rate of the sample.

Typical Standard Curve



Specificity

cAMP: 100%

AMP: <0.0001%

ATP: <0.0001%

cGMP: <0.0001%

GMP: <0.0001%

GTP: <0.0001%

cUMP: <0.0001%

CTP: <0.0001%

Precautions

1. Please store this kit at the corresponding temperature according to the instructions for use, and store the standard, antibody and enzyme conjugate at -80°C. Avoid repeated freezing and thawing.
2. Please place the kit to equilibrate to room temperature before use (at least 30 minutes).
3. Please tighten the bottle cap after using the reagent to avoid volatilization or cross contamination.
4. After the sample is processed, the pH value should be neutral after mixing with an equal amount of neutralizing solution, and the sample to be tested should be a transparent colorless liquid.
5. Immediately put the unused microplate into the packaging bag, seal it tightly, and store it at 4°C.
6. If the reagent sticks to the wall of the tube, please centrifuge it to the bottom of the tube for a while and dilute it as needed.
7. All prepared reagents should be added to the microplate within 15 minutes as much as possible. After adding all the reagents, shake the enzyme label plate slightly to mix the reagents, and do not splash.
8. Please equilibrate the color development solution to room temperature in advance, and color development at room temperature for 8~10 minutes. Do not shake it.
9. When you see a clear competition curve, you can stop the reaction. After adding the stop solution, please shake the microplate gently until the reaction is completely stopped and read the result immediately.

10. When using 0.1M HCl to treat the sample, try to keep the cAMP concentration in the sample within the detection range of the kit.
11. In order to ensure the accuracy and reliability of the test, please repeat each sample at least 2 times.

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