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

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cAMP ELISA Kit

(Catalog # E4715-100; 96 assay; Storage at 4ºC)

I. Introduction:

Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are second messenger molecules important in regulating intracellular energy metabolism via actions on specific protein kinases. Both are highly enriched in the CNS; cAMP is involved in higher cortical functions, while cGMP plays a role in phototransduction. Most studies suggest that normal lumbar CSF (Cerebrospinal fluid) in adults contains 15–30 nmol/l of cAMP and one study reports that intraventricular levels may be 2- to 3-fold than lumbar levels. cAMP is a derivative of adenosine triphosphate (ATP) and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway. cAMP involvement in mechanisms of transmembrane regulation of cell metabolism, differentiation, proliferation in malignant growth is promising as protecting the CT and regulating body homeostasis. This BioVision's cAMP ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with cAMP. During the reaction, cAMP in the sample or standard competes with a fixed amount of cAMP on the solid phase supporter for sites on the Biotinylated Detection Ab specific to cAMP. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of cAMP in the samples is then determined by comparing the OD of the samples to the standard curve.

II. Applications:

- cAMP ELISA kit can be used for in vitro quantitative determination of cAMP concentrations in serum, plasma and other biological fluids.
- **Sensitivity:** 0.94 ng/mL
- **Detection Range:** 1.56-100ng/mL
- **Specificity:** This kit recognizes cAMP in samples. No significant cross-reactivity or interference between cAMP and analogues was observed.
- Coefficient of variation is <10%.

III. Sample Type:

- Plasma, Serum, Other biological fluids

IV. Kit Contents:

Components	E4715-100	Part Number	Storage Temp
Micro ELISA Plate	8 wells ×12 strips	E4715-100-1	-20°C
Standard	2 vials	E4715-100-2	-20°C
Biotinylated Detection Antibody (100×)	120 µl	E4715-100-3	-20°C
HRP Conjugate (100×)	120 µl	E4715-100-4	-20°C
Standard & Sample Diluent	20 ml	E4715-100-5	4°C
Biotinylated Detection Ab Diluent	14 ml	E4715-100-6	4°C
HRP Conjugate Diluent	14 ml	E4715-100-7	4°C
Wash Buffer (25×)	30 ml	E4715-100-8	4°C
Substrate Reagent	10 ml	E4715-100-9	4°C
Stop Solution	10 ml	E4715-100-10	4°C
Plate Sealer	5	E4715-100-11	

V. User Supplied Reagents and Equipment:

- Microplate reader with 450 nm wavelength filter
- Deionized or distilled water

VI. Storage Conditions and Reagent Preparation:

Storage and Handling:

An unopened kit can be stored at 4°C for 1 month. **If the kit is not used within 1 month, store the items separately according to the recommended conditions once the kit is received.**

1. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. **Note:** if crystals have formed, incubate it in a 40°C water bath and mix it gently until the crystals have completely dissolved.

2. Biotinylated Detection Antibody: Calculate the required amount before the experiment (50µL/well). Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent

Bring all reagents to room temperature (18~25°C) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.

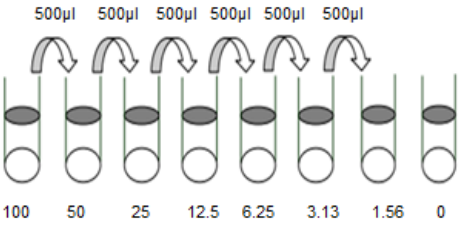
3. HRP Conjugate: Calculate the required amount before the experiment (100 µl/well). Dilute the 100×Concentrated HRP Conjugate to

1×working solution with Concentrated HRP Conjugate Diluent.

4. Standard Preparation:

Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 100 ng/mL. Prepare serial dilutions as needed. Suggested standard points are: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/mL.

Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube. Pipette 500 µl of the 100 ng/mL working solution to the first tube and mix up to produce a 50 ng/mL working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.



5. Sample Preparation:

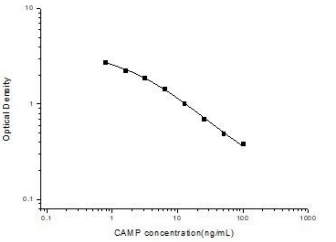
Note: Samples should be assayed within 7 days when stored at 4℃, otherwise aliquot and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles

- **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and endotoxin free.
- **Plasma:** Collect plasma using EDTA-Na2 as anticoagulant. Centrifuge samples for 15 min at 1000× g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. **Hemolysed samples are not suitable for ELISA assay!**
- **Saliva:** Remove particulates by centrifugation for 10 minutes at 4000×g at 2-8°C. Collect the supernatant to carry out the assay. Recommend to use fresh saliva samples.
- **Urine:** Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at 1000×g at 2-8°C. Collect the supernatant to carry out the assay.

VII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 50 µl of each **standard or samples** into appropriate wells.
2. Immediately add 50 µl of **Biotinylated Detection Antibody** working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. **Note:** solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
3. Aspirate the solution from each well add 350 µl of **1x wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 µl of **HRP Conjugate** working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 2.
6. Add 90 µl of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a microplate reader set to 450 nm.
9. **Calculation:** Determine the average of the duplicate readings for each standard and samples. Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution. The actual concentration is the concentration obtained by calculation multiplied by the dilution factor.



Conc. (ng/ml)	100	50	25	12.5	6.25	3.13	1.56	0
OD	0.384	0.493	0.695	1.02	1.455	1.904	2.267	2.763

VIII. Related Products:

cAMP Direct Immunoassay Kit (Colorimetric) (K371)
Anti-cAMP Antibody (4H2B6) (A1286)

FOR RESEARCH USE ONLY! Not to be used on humans.