cAMP ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF cAMP CONCENTRATIONS IN SERUM, PLASMA, CELL CULTRES AND URINE

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

ELISA NAME	**cAMP ELISA KIT**
Catalog No.	SK00716-06
Lot No.	
Formulation	96 T
Standard range	1-10000 pmol/mL
Dynamic range	1-1000 pmol/mL
Sensitivity	0.05 pmol/ml
Sample Volume	50 µl per well
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA plasma, cell culture
Specificity	cAMP
Calibration	cAMP
Intra-assay Precision	6 – 8%
Inter-assay Precision	12 – 14%
Storage	2 – 8° C for a few weeks, -20° C ~ -70° C for longer storage.
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

Adenosine 3', 5' cyclic monophosphate (cAMP) ELISA Kit contains the necessary components required for the quantitative measurement of natural cAMP from serum, plasma, urine and cell culture supernatant in a competitive enzyme immunoassay technique format.

This immunoassay contains cAMP and the monoclonal antibody raised against cAMP. Results from this immunoassay have shown to accurately quantify natural cAMP of human and rat samples. If the samples has mouse IgG Fc, it cannot be tested by this cAMP ELISA kit because the plate was coated with anti mouse IgG Fc antibody.

ASSAY OVERVIEW

cAMP ELISA employs the quantitatively competitive enzyme immunoassay technique in which cAMP present in samples compete with a fixed amount of cAMP-HRP conjugate for sites on an antibody specific against cAMP. Following standard and sample binding to the antibody for 90 minutes, the cAMP-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of cAMP bound in the initial step. The sample values are then read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

Description	Code	Quantity
AMIGFC-Microplate - 96 well microplate pre-coated with polyclonal anti mouse IgG Fc purified IgG.	**AMIGFC**	**1 plate**
cAMP Standard – 100 nmol/ml of cAMP in a buffered protein base with preservative.	**716-06-01**	**1 vial**
cAMP-HRP Solution Concentrate - 60 µL/vial, 100-fold concentrate of cAMP HRP conjugate with preservative.	**716-06-02**	**1 vial**
cAMP Antibody Concentrate – 100 µl/vial, 60-fold concentrate of monoclonal purified IgG against cAMP with preservative; lyophilized.	**716-06-03**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to a few weeks. For longer storage, unopened Standard, Antibody Concentrate and HRP Solution Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Cell Cultures Supernatant – Use serum free media for cell culture because fetal bovine serum has cAMP.

If the samples contain any mouse IgG or its Fc, please let the samples go through Protein A or Protein G affinity column (Order code No. ProA-001 or ProG-001) to remove off all mouse IgG Fc before sample test.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

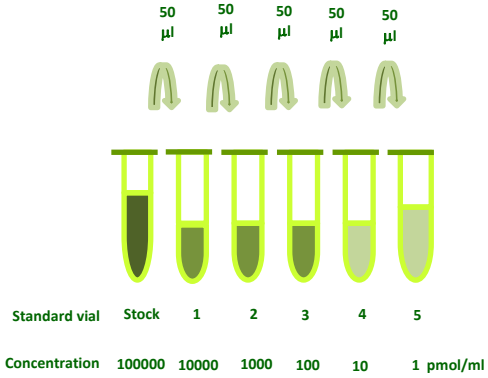
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

cAMP Standard - This stock solution has 100000 pmol/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of Dilution Buffer into tubes #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **10000 pmol/mL** standard serves as the high standard.

Tube	Standard	Dilution Buffer	Concentration
Stock	liquid	0	100000 pmol/ml
# 1	50µl of stock	450µl	10000 pmol/ml
# 2	50µl of 1	450µl	1000 pmol/ml
# 3	50µl of 2	450µl	100 pmol/ml
# 4	50µl of 3	450µl	10 pmol/ml
# 5	50µl of 4	450µl	1 pmol/ml



cAMP Antibody Concentrate - Transfer 100 µL of cAMP Antibody Concentrate (60-fold) to 5.9 mL of Dilution Buffer to prepare **1x Antibody Solution**.

cAMP-HRP Solution Concentrate -Transfer 60 µL of cAMP-HRP Solution Concentrate (100-fold) to 5.94 mL of Dilution Buffer to prepare **1x cAMP-HRP Solution (protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR HRP SOLUTION INTO BLANK WELLS.***
4. Set two wells as Total Binding. Add 50 µl per well of **Dilution Buffer**.
5. Add 50 µL per well of **Standard dilutions** from #1 to #5 (reverse order of serial dilution) to the appropriate wells. Add 50 µL per well of **samples** into other wells.
6. Add 50 µL per well of **1x Antibody Solution** into total binding, standard dilutions and sample wells. Cover with plate sealer and incubate on microplate shaker (250 - 300rpm) at room temperature for 90 minutes. ***Note: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.***
7. Add 50 µL per well of **1x cAMP-HRP Solution** into total binding, standard dilutions and sample wells. Cover with plate sealer and incubate on microplate shaker at room temperature for 60 minutes. **Note:** DO NOT ADD cAMP-HRP Solution to Blank wells. **Protect from light**.
8. Aspirate wells and wash 4 times with 300 µl of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for 17-20 minutes on microplate shaker at room temperature. **Protect from light**.
12. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. It is recommended to add the stop solution when the total Binding or the lowest standard has developed a dark blue color.
13. Determine the optical density of each well. Set the microplate reader to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Well	OD450 reading	Standard (pmol/mL)
Blank	0.042	
Total Binding	1.358	0
Standard 5	1.279	1
Standard 4	1.127	10
Standard 3	0.638	100
Standard 2	0.187	1000
Standard 1	0.072	10000

SPECIFICITY

Item	Cross-reactivity
cAMP	100%
cGMP	0.12%
ATP	0.37%

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 50 µL of standard dilutions and samples to the wells. Add 50 µL of 1x Antibody solution to each well. Incubate 90 minutes on the plate shaker at RT. **Do not wash or aspirate. Proceed to next step.**

Add 50 µL 1x cAMP-HRP Solution to each well. Incubate 60 minutes on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 µL Substrate Solution to each well. Incubate 17-20 min on the plate shaker at RT. **Protect from light.**

Add 100 µL Stop Solution to each well. Read at 450nm.