EAGLE
BIOSCIENCES

cAMP Urine
ELISA Assay Kit

Catalog Number:
CMP14-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 3.0 (08 AUG 24)

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INTENDED USE

The Eagle Biosciences cAMP Urine ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative measurement of cAMP (cyclic adenosine-3', 5'-monophosphate) in human urine by an enzyme immunoassay. The Eagle cAMP Urine ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The cAMP Urine ELISA is a competitive immunoassay. Competition occurs between cAMP present in calibrators, controls, specimen samples and an enzyme-labeled antigen (HRP conjugate) for a limited number of anti-cAMP antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of cAMP present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of cAMP in specimen samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    c. Wear protective clothing and disposable gloves.
    d. Wash hands thoroughly after performing the test.
    e. Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or urine pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.

14. The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
16. Samples values above the measuring range of the kit may be reported as >30,000 pmol/mL. If further dilution and retesting is required, only the sample diluent may be used to dilute urine samples. The use of any other reagent may lead to false results.
17. Avoid microbial contamination of reagents.
18. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
19. To prevent the contamination of reagents, do not pour reagents back into the original containers.
20. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
21. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
22. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
23. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solution.
24. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
25. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
26. Do not reuse the microplate wells, they are for SINGLE USE only.
27. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
28. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

LIMITATIONS

1. This kit is intended for research use only and should not be used as a diagnostic tool.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDOUS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

Specimen Collection & Storage

Approximately 0.01 mL of urine is required per duplicate determination.

Either spontaneous urine or 24-hour urine may be used in this assay

To prevent dilute urine samples, avoid drinking excessive amounts of fluids prior to urine collection. Failure to do so could result in abnormally low cAMP levels.

Spontaneous Urine Collection: Collect 4-5 mL or urine into an appropriately labeled container

24-Hour Urine Collection: Collect urine into a specimen collection container over a 24-hour period (24-hour urine). Add 4 mL of 12 N concentrated HCl per liter as a preservative.

Storage: Urine specimen samples may be stored at room temperature (20-25^{o}C) for up to 3 days, at 2-8^{o}C for up to 7 days or at -20^{o}C or lower for up to 3 months.

Avoid more than 3 freeze-thaw cycles.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Specimen Pre-Treatment & Storage

After stored urine specimens have been brought to room temperature, inspect each sample to ensure that they are free from precipitates prior to undergoing the specimen pre-treatment. If there are precipitates present, vortex and centrifuge the sample. Use the clear supernatant for the pre-treatment steps stated below.

All urine specimens must be diluted 1:100 in the provided Assay Buffer before being used in the test. Follow the specimen pre-treatment procedure as stated below for each specimen that is to be tested:

1. Pipette 0.99 mL of the Assay Buffer into a new polypropylene microcentrifuge tube.
2. Pipette 10 µL of the urine specimen into the tube from step 1 that contains 0.99 mL of assay buffer.
3. Close the tube and label it with specimen identification information.
4. Mix the contents of the tube by vortexing.

Do not pre-treat the calibrators and kit controls; they are provided in a ready to use format
Note: Different volumes of the Assay Buffer and urine specimen may be used provided that the required 1:100 ration is maintained (1 part urine specimen to 99 parts Assay Buffer).

Pre-treated urine specimens must be assayed on the same day as they were prepared. Do not store pre-treated urine specimens beyond this time limit.
Consider all human specimens as possible biohazardous materials.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 10 µL, 40 – 150 µL and 0.99 mL.

2. Calibrated multi-channel pipettes to dispense 50 µL, and 150 µL.
3. Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Disposable pipette tips
6. Distilled or deionized water
7. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater
8. Polypropylene or HDPE tubes for sample dilution (e.g. polypropylene microcentrifuge tubes).
9. Centrifuge
10. 12 N Hydrochloric acid (HCl) (may be required, refer to SPECIMEN COLLECTION & STORAGE section).

REAGENTS PROVIDED

1. **Microplate**

Contents:	One cAMP polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three months.

2. **HRP Conjugate Concentrate Lyophilized**

Contents:	One bottle containing lyophilized cAMP-Horse Radish Peroxidase (HRP) conjugate in a stabilizing buffer with a non-mercury preservative.
Format:	Lyophilized and Concentrated; Requires Preparation
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening and Reconstitution: Stable for **three months**. Following Preparation: The HRP Conjugate Working Solution is stable for **8 hours** at room temperature.
Reconstitution:	Reconstitute the lyophilized HRP Conjugate by adding 0.5 mL of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 10 minutes. Mix gently without foaming before use.
Preparation of Biotin Conjugate Working Solution:	**Dilute 1:51 Before Use** Dilute the **reconstituted** HRP Conjugate **1:51** in **Conjugate Diluent** (e.g., 40 µL of reconstituted conjugate in 2 mL of conjugate diluent). If the whole plate is to be used dilute 120 µL of the reconstituted HRP Conjugate in 6 mL of conjugate diluent.

3. **Calibrator A-F**

Contents:	Six bottles of calibrator containing specified cAMP concentrations. Stabilizing buffer with a non-mercury preservative. Prepared by spiking buffer with defined

quantities of cAMP

Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Concentrations: 0, 3, 10, 30, 100, 300 pmol/mL.

Format: Ready to Use

Volume: 1.0 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three months.

4. **Control 1-2**

Contents: Two bottles of control containing different cAMP concentrations. Stabilizing buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of cAMP.

Refer to the QC certificate for the target values and acceptable ranges.

Format: Ready to Use

Volume: 1.0 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for three months.

5. **Conjugate Diluent**

Contents: One bottle containing a stabilizing buffer with a non-mercury preservative.

Format: Ready to Use

Volume: 10 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for 8 weeks.

6. **Sample Diluent**

Contents: One bottle containing a stabilizing buffer with a non-mercury preservative.

Format: Ready to Use

Volume: 50 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 8 weeks.

7. **TMB Substrate**

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Format:	Ready to Use
Volume:	18 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 8 weeks.

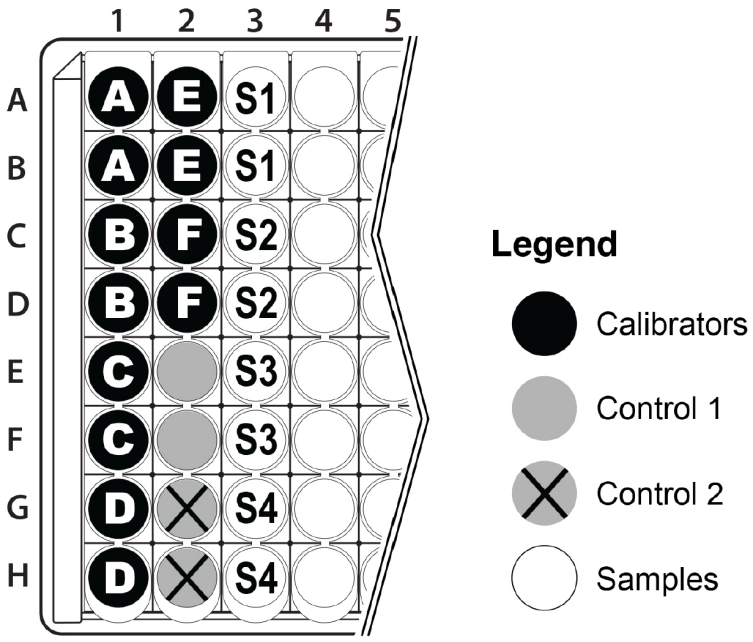
8. **Stopping Solution**

Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	8 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 8 weeks.
Safety:	Refer to product SDS

9. **Wash Buffer Concentrate**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 8 weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.
Preparation of Wash Buffer Working Solution:	Dilute 1:10 Before Use Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Specimen Pre-Treatment: All specimens that will be tested must be pre-treated before being tested (see SPECIMEN PRE-TREATMENT & STORAGE section). Do not pre-treat the calibrators and kit controls as they are provided ready to use.

All kit components, controls and specimen samples must reach room temperature prior to use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature **mix** gently by inversion
2. **Prepare** the HRP Conjugate Working Solution and Wash Buffer Working Solution (see REAGENTS PROVIDED, HRP CONJUGATE CONCENTRATE LYOPHILIZED and WASH BUFFER CONCENTRATE section).
3. **Prepare** all specimen samples that will be tested. Refer to section SPECIMEN PRE-TREATMENT & STORAGE
4. **Plan** the microplate wells to be used for calibrators, controls and samples. See section RECOMMENDED ASSAY LAYOUT. Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
5. **Pipette 100 µL** of each calibrator, control, and pre-treated specimen sample into assigned wells.
6. **Pipette 50 µL** of the HRP Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
7. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature (no shaking) for **60 minutes.**

8. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manually: Perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
9. **Pipette 150 µL** of the Streptavidin HRP Conjugate into each well (the use of a mutli-channel pipette is recommended).
10. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature (no shaking) for **20 minutes.**
11. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
12. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with the immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. The final concentration of the urine specimen samples must take into account 1:100 dilution that was performed during the specimen pre-treatment step. Calculate the final plasma specimen cAMP concentration using the following formula:

   Final urine specimen cAMP concentration =
   Concentration calculated from calibrator curve x **100** (dilution factor)

   Example:
   If the urine sample concentrations calculated from the calibrator curve was 10 pmol/mL, then the final concentration of cAMP in the plasma specimen sample = 10 pmol/mL x **100** = 1000 pmol/mL.

   Do not perform any calculations to samples that did not undergo the specimen pre-treatment (dilution) step (e.g. kit controls).

5. If a urine sample reads more than 300 pmol/mL (30,000 pmoL/mL considering the dilution factor of 1:100), then dilute the 1:100 diluted sample (normal dilution) up to a 1:10 dilution, using the supplied Assay Buffer. The result obtained must be multiplied by the dilution factor that was used.

   Example:

If the 1:100 diluted urine specimen (normal dilution) is further diluted 1:10 and produces a result of 200 pmol/mL, then the final urine specimen cAMP concentration = 200 pmol/mL x **100** x **10 =** 200,000 pmol/mL

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator / OD of calibrator A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

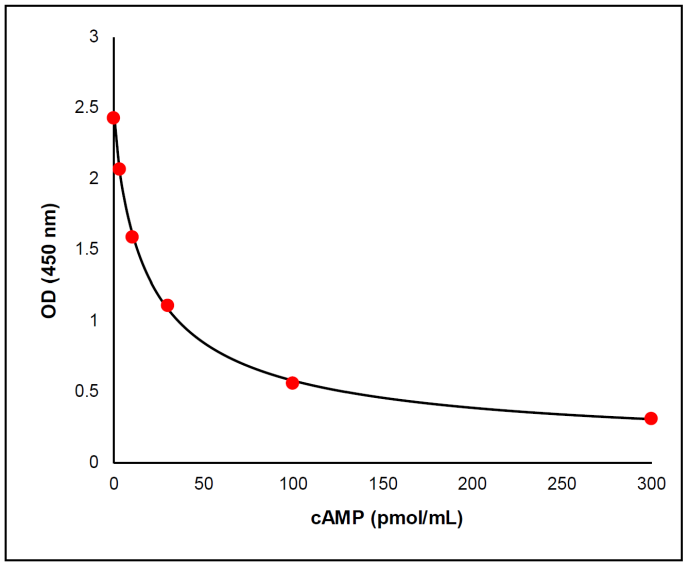
TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.431	100	0
B	2.073	82	3
C	1.588	65	10
D	1.113	46	30
E	0.564	23	100
F	0.313	13	300
Unknown	1.253	-	21.3

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.