Diagnostics Biochem Canada Inc.
Manufacturing Innovative IVD for the World
Since 1973

cAMP Plasma ELISA

REF CAN-AMP-4170	**RUO**
Effective Date: August 17, 2023	Version: RUO-1.0

1. INTENDED PURPOSE & USE

For the quantitative measurement of cAMP (cyclic adenosine-3',5'-monophosphate) in human EDTA plasma by an ELISA (Enzyme-Linked Immunosorbent Assay).

For Research Use Only. Not for use in diagnostic procedures.

2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This kit is intended for research use only and is not to be used for any diagnostic procedures.

3. PRINCIPLE OF THE TEST

The cAMP Plasma ELISA is a competitive immunoassay. Competition occurs between cAMP present in calibrators, controls, specimen samples and an enzyme-labelled 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-coloured 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 colour 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.

4. 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:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - 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 EDTA plasma 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 colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored plasma.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as > 500 pmol/mL. If further dilution and retesting is required, only the sample diluent may be used to dilute EDTA plasma samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. 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.
23. 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.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. 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.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. 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.

5. SAFETY CAUTIONS AND WARNINGS

5.1 BIOHAZARDS

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.

5.2 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.

6. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

6.1 Specimen Collection & Storage

Approximately 0.05 mL of EDTA plasma is required per duplicate determination.

1. Collect 4–5 mL of venous blood into an appropriately labelled EDTA plasma tube.
2. Mix the tube by inverting several times.
3. Centrifuge the sample at room temperature for 15 minutes at 2000 RCF.
4. Transfer the plasma sample into a new labelled storage tube.

Plasma samples may be stored at 2-8°C for up to 2 days or at -20°C or lower for up to 3 months. Avoid more than two freeze-thaw cycles.

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

6.2 Specimen Pre-Treatment & Storage

All EDTA plasma specimens must be diluted 1:5 in the provided Sample Diluent 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 0.2 mL of the **Sample Diluent** into a new polypropylene microcentrifuge tube.
2. Pipette 50 µL of the plasma specimen into the tube from step 1 that contains 0.2 mL of Sample Diluent.
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 controls; they are provided in a ready-to-use format.

Note: Different volumes of the Sample Diluent and specimen sample may be used provided that the required 1:5 ratio is maintained (1 part EDTA plasma sample to 4 parts Sample Diluent).

Pre-treated plasma specimens (1:5 dilution used in the test) must be assayed on the same day. Do not store diluted specimens beyond this time limit.

Consider all human specimens as possible biohazardous materials.

7. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 40 – 200 µL.
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 capable of 2000 RCF.
10. Vortex mixer.

8. REAGENTS PROVIDED

1. MPL — 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 8 weeks.

2. HRP | CONJ | CONC | LYO — 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 8 weeks. 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 HRP Conjugate Working Solution:	**X51** — **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. CAL | A – F — 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, 1.5, 4, 10, 40, 100 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 8 weeks.

4. CONTROL | 1 – 2 — 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 8 weeks.

5. CONJ | DIL — 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. SMP | DIL — 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 | SUB — 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.

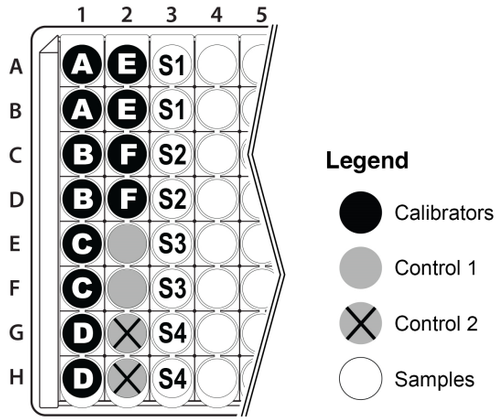
8. STOP — Stopping Solution

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.  **Warning**

9. WASH | BUFF | CONC — 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:	**X10** **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.

9. RECOMMENDED ASSAY LAYOUT



10. ASSAY PROCEDURE

Specimen Pre-Treatment:

All specimens that will be tested must be pre-treated before being tested (see section *6.2. Specimen Pre-Treatment & Storage*). 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 section *8. Reagents Provided* section, *2. HRP Conjugate Concentrate Lyophilized* and *9. Wash Buffer Concentrate*).
3. **Prepare** all specimen samples that will be tested. Refer to section *6.2. Specimen Pre-Treatment & Storage*.
4. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section *9. 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: For manual washing, 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 TMB Substrate into each well (the use of a multi-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.

11. CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with 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 EDTA plasma specimen samples must take into account the 1:5 dilution that was performed during the specimen pre-treatment step. Calculate the final plasma specimen cAMP concentration using the following formula:

   ***Final plasma specimen cAMP concentration* =**
   Concentration calculated from calibrator curve x **5** (dilution factor).

   Example:
   If the plasma sample concentration calculated from the calibrator curve was 5 pmol/mL, then the final concentration of cAMP in the plasma specimen sample = 5 pmol/mL x **5** = 25 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 plasma sample reads more than 100 pmol/mL (500 pmol/mL considering the dilution factor of 1:5), then dilute the 1:5 diluted sample (normal dilution) up to a 1:5 dilution, using the supplied Sample Diluent. The result obtained must be multiplied by the dilution factor that was used.

   Example:
   If the 1:5 diluted plasma specimen (normal dilution) is further diluted 1:5 and produces a result of 20 pmol/mL, then the final plasma specimen cAMP concentration = 20 pmol/mL x **5** x **5** = 500 pmol/mL.

12. 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.

13. TYPICAL DATA

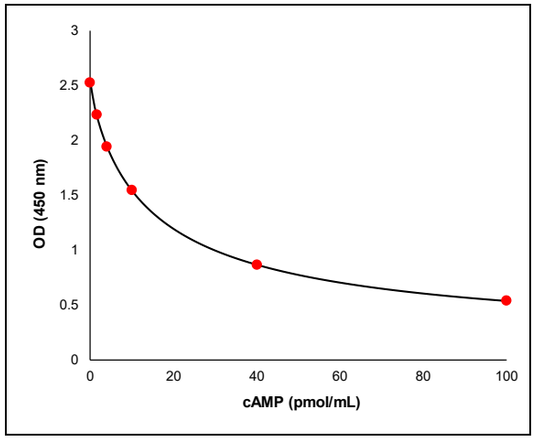
13.1 TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pmol/mL)
A	2.528	100	0
B	2.234	88	1.5
C	1.946	77	4
D	1.546	61	10
E	0.870	34	40
F	0.541	21	100
Unknown	2.003	-	3.4

13.2 TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



14. SYMBOLS GLOSSARY

Symbol	Definition	Symbol	Definition
REF	Catalogue number		Manufacturer
LOT	Batch code		Date of manufacture
IVD	In vitro diagnostic medical device		Biological risks
UDI	Unique Device Identifier		Consult instructions for use
X #	Dilute 1:# Before Use	Rx ONLY	Prescription only: Device restricted to use by or on the order of a physician
QTY	Quantity		Keep away from sunlight
	Use-by date	EC REP	Authorized representative in the European Community/ European Union
	Do not re-use		Temperature limit
	Caution		Contains sufficient for <n> tests
LYO	Lyophilized	RUO	For Research Use Only. Not for use in diagnostic procedures.

The definitions of symbols used for kit component names are described in the *Reagents Provided* section.

15. CHANGE HISTORY

Previous Version:	-	**New Version:**	RUO-1.0 Build: *v1.4D*
Changes:	-		

16. GENERAL INFORMATION

Diagnostics Biochem Canada (DBC) Inc.
384 Neptune Crescent
London, Ontario, Canada N6M 1A1
Tel: (519) 681-8731
Fax: (519) 681-8734
e-mail: dbc@dbc-labs.com
www.dbc-labs.com

Product Complaints

In the case of product complaints, the user shall submit in writing to the distributor or manufacturer a description of the complaint and provide accompanying data and/or information.

Warranty

DBC guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

Limitation of Liability

DBC liability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.