Version 2c Last updated 12 March 2026

ab290713
cAMP Assay Kit (Competitive ELISA)

For quantitative detection of Cyclic AMP Complete in cells and tissue treated with 0.1M HCL, in addition to Plasma, Saliva, Serum and Cell Culture Supernatants.

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

Table of Contents

1. Overview

Abcam's Cyclic AMP (cAMP) *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) is a colorimetric competitive immunoassay kit for the quantitative determination of extracellular cAMP diluted in buffer or intracellular cAMP in cells or tissues lysed in 0.1 M HCl.

A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-cAMP antigen and a polyclonal rabbit antibody specific to cAMP. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of cAMP captured in the plate.

Adenosine 3', 5'-cyclic monophosphate (cyclic AMP) is one of the most important "second messengers" involved as a modulator of physiological processes. Cyclic AMP Complete is also involved in regulating neuronal, glandular, cardiovascular, immune and other functions. A number of hormones are known to activate Cyclic AMP Complete through the action of the enzyme adenylate cyclase which converts ATP to Cyclic AMP Complete. These hormones include a variety of anterior pituitary peptide hormones such as corticotrophin (ACTH), glucagon, calcitonin, thyroid stimulating hormone (TSH), and luteinizing hormone (LH). Because Cyclic AMP Complete has been shown to be involved in the cardiovascular and nervous systems, immune mechanisms, cell growth and differentiation, and general metabolism, there remains considerable interest in the measurement of intracellular Cyclic AMP Complete in tissues and cell cultures. The investigation of Cyclic AMP Complete may help to provide a clearer understanding of the physiology and pathology of many disease states.

2. Protocol Summary

Prepare all reagents and samples as instructed

Add standards and samples to appropriate wells.

Add prepared labeled AP-conjugate to appropriate wells.

Add Cyclic AMP Complete antibody to appropriate wells. Incubate at room temperature.

Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the Alkaline Phosphatase Conjugate and Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 wells	+4°C
Cyclic AMP Complete Alkaline Phosphatase Conjugate	5 mL	-20°C
Cyclic AMP Complete Antibody	5 mL	+4°C
Cyclic AMP Complete Standard	500 µL	-20°C
20X Wash Buffer Concentrate	27 mL	+4°C
pNpp Substrate	20 mL	+4°C
Stop Solution	5 mL	+4°C
Acetylation kit - Triethylamine	2 mL	+4°C
Acetylation kit - Acetic Anhydride	1 mL	+4°C
Assay Buffer 2	27 mL	+4°C
0.1M HCl	27 mL	+4°C
Neutralizing Reagent	5 mL	+4°C
Plate Sealer	1 unit	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- Eppendorf tubes.
- Microplate Shaker.
- Absorbent paper for blotting.
- Washing buffer.
- Triton X-100 (optional for sample preparation).
- Optional (for tissue samples): Liquid nitrogen, mortar & pestle, and concentrated HCl.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
- The standard should be handled with care due to the known and unknown effects of the antigen.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Cyclic AMP Complete Alkaline Phosphatase Conjugate

Allow the Cyclic AMP Complete Alkaline Phosphatase Conjugate to warm to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

9.3 Acetylation Reagent (optional)

Prepare the Acetylating Reagent by adding 0.5 mL of Acetic Anhydride to 1 mL of Triethylamine. Note that this volume is sufficient to add to 30 mL of diluted standards and samples. Use the prepared reagent within 60 minutes of preparation.

Discard any unused portion of the Acetylating Reagent.

10. Standard Preparation – Non-Acetylated Format

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Diluted standards should be used within 60 minutes of preparation.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 For samples in:

10.1.1 **Plasma**, **Serum** and **saliva**: dilute the Cyclic AMP standard by Assay Buffer 2.

10.1.2 **Culture supernatant:** dilute standards with the same non-conditioned media.

10.1.3 **Cell lysates** and **Tissue** samples prepared in 0.1M HCL: dilute standards with the supplied 0.1M HCL.

10.2 Allow the 2,000 pmol/mL Cyclic AMP Stock Standard solution to equilibrate to room temperature. Avoid repeated freeze-thaw cycles.

10.3 Label five tubes with numbers #1 – #5.

10.4 Add 900 µL of appropriate diluent (Assay Buffer 2 or Tissue Culture Media) to tube #1.

10.5 Add 750 µL of appropriate diluent to tubes #2 - #5.

10.6 Prepare a 200 pmol/mL **Standard 1** by adding 100 µL of 2,000 pmol/mL Stock Standard to tube #1. Vortex thoroughly.

10.7 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.8 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.9 Using the table below as a guide, repeat for tubes 4 and 5.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pmol/mL)	Final Conc. (pmol/mL)
1	100 µL Standard	900	2,000	200
2	250 µL Standard #1	750	200	50
3	250 µL Standard #2	750	50	12.5
4	250 µL Standard #3	750	12.5	3.13
5	250 µL Standard #4	750	3.13	0.78

11. Standard Preparation – Acetylated Format (optional)

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Acetylated standards should be used within 30 minutes of preparation.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

11.1 For samples in:

11.1.1 **Serum** and **saliva**: dilute the Cyclic AMP standard by Assay Buffer 2.

11.1.2 **Culture supernatant:** dilute standards with the same non-conditioned media.

11.1.3 **Cell lysates** and **Tissue** samples prepared in 0.1M HCL: dilute standards with the supplied 0.1M HCL.

11.2 Allow the 2,000 pmol/mL Cyclic AMP Stock Standard solution to equilibrate to room temperature. Avoid repeated freeze-thaw cycles.

11.3 Label five tubes with numbers #1 – #5.

11.4 Add 990 µL of appropriate diluent (Assay Buffer 2 or Tissue Culture Media) to tube #1.

11.5 Add 750 µL of appropriate diluent to tubes #2 - #5.

11.6 Prepare a 20 pmol/mL **Standard 1** by adding 10 µL of 2,000 pmol/mL Stock Standard to tube #1. Vortex thoroughly.

11.7 Prepare Standard 2 by transferring 250 µL from Standard 1 to tube #2. Vortex thoroughly.

11.8 Prepare Standard 3 by transferring 250 µL from Standard 2 to tube #3. Vortex thoroughly.

11.9 Using the table below as a guide, repeat for tubes #4 and #5.

11.10 Acetylate all standards by adding 10 µL of the Acetylating Reagent for each 200 µL of the standard. Add the Acetylating Reagent directly to the diluted standard or sample and vortex immediately after the addition of the Acetylating Reagent.

Δ Note If acetylating standards, then samples must be acetylated in the same format by adding 10 µL of the Acetylating Reagent for each 200 µL of the sample.

11.11 Add 1 mL of Assay Buffer 2 into the B_O tube, followed by 50 µL of the Acetylating Reagent. Use as directed in the Assay Procedure.

11.12 If acetylating standards, then samples must be acetylated in the same format by adding 10 µL of the Acetylating Reagent for each 200 µL of the sample.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pmol/mL)	Final Conc. (pmol/mL)
1	10 µL Standard	990	2,000	20
2	250 µL Standard #1	750	20	5
3	250 µL Standard #2	750	5	1.25
4	250 µL Standard #3	750	1.25	0.312
5	250 µL Standard #4	750	0.312	0.078

12. Sample Preparation

- Samples must be stored frozen at or below -20ºC to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.
- Treatment of cells and tissue with HCl will stop endogenous phosphodiesterase activity and allow for the direct measurement of these samples in the assay without evaporation or further processing.
- Samples containing rabbit IgG will interfere with the assay. EDTA plasma may precipitate during acetylation.
- Biological fluids should be diluted in Assay Buffer 2 and run directly in the assay. When using the non-acetylation protocol, a minimum 1:16 dilution is required for plasma, a 1:64 dilution for serum, a 1:16 dilution for saliva. When using the acetylation protocol, a minimum 1:64 dilution is required for both plasma and serum and a 1:2 dilution for saliva. Culture supernatant, diluted in both assay buffer and tissue culture media, has also been validated for use in this kit. When using the non-acetylation protocol, neat culture supernatant can be used. When using the acetylation protocol, a minimum 1:4 dilution is required. These are the minimum dilutions required to remove matrix interference of these samples.
- Culture supernatants may be run directly in the assay provided the same non-conditioned media is used as the standard diluent.
- Please note that some samples may contain high levels of Cyclic AMP Complete and additional dilution may be required. Samples with low levels of Cyclic AMP Complete may be assayed in the acetylated format or the samples may be concentrated.

12.1 Protocol for Cell Lysates:

The concentration of cells used must be optimized for the specific cell line and treatment conditions. Cells may be grown in typical containers such as Petri dishes, culture plates (e.g., 48-well, 12-well, or 96-well), culture flasks, etc. Some cells are particularly hardy (e.g., bacteria) and may require the addition of 0.1 to 1% Triton X-100 to the 0.1M HCl for enhanced lysis. If Triton X-100 is added to samples it should also be added to the standard dilution as a modest increase in optical density may occur.

12.1.1 Pellet suspension cells and aspirate the media. Treat cells with 0.1M HCl. A general starting concentration of 1 x 10^6 cells per mL of 0.1M HCl is recommended. Remove the media from adherent cells and add enough 0.1M HCl to cover the bottom of the plate. Avoid over-diluting the sample with an excessive volume of HCl. Please note that the culture media may be saved and assayed separately, if desired.

12.1.2 Incubate the cells in 0.1M HCl for 10 minutes at room temperature.

12.1.3 Inspect the cells under a microscope to ensure uniform lysis. Continue incubating for an additional 10 minutes, if necessary.

12.1.4 Centrifuge ≥600 x g to pellet the cellular debris.

12.1.5 The supernatant may be assayed immediately or stored frozen for later analysis.

Δ Note Standards must be diluted in 0.1 M HCl and Neutralizing Reagent used.

12.2 Protocol for Tissue Samples:

12.2.1 After collection, tissue samples should be flash frozen in liquid nitrogen. If analysis cannot be carried out immediately, store tissue at -80°C.

12.2.2 Grind frozen tissue to a fine powder under liquid nitrogen in a stainless steel mortar.

12.2.3 When liquid nitrogen has evaporated, weigh the frozen tissue and homogenize in 10 volumes of 0.1M HCl (e.g., 0.1 g of tissue should be homogenized in 1 mL of 0.1M HCl).

12.2.4 Centrifuge ≥600 x g to pellet the debris (~10 minutes).

12.2.5 The supernatant may be further diluted in the 0.1M HCl provided and run directly in the assay or stored frozen for later analysis.

Δ Note Standards must be diluted in 0.1 M HCl and Neutralizing Reagent used.

13. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

Recommended plate layout

	1	**2**	**3**	**4**
A	B_s	Std 1	Std 5	etc
B	B_s	Std 1	Std 5	etc
C	TA	Std 2	Sample 1	
D	TA	Std 2	Sample 1	
E	NSB	Std 3	Sample 2	
F	NSB	Std 3	Sample 2	
G	B_0	Std 4	Sample 3	
H	B_0	Std 4	Sample 3	

Plate layout shows controls, blanks and standards required for each assay. Use additional strips of wells to assay all your samples.

Key:

B_s = Blank; contains substrate only.

TA = Total Activity; contains conjugate (5 µL) and substrate.

NSB = Non-specific binding; contains standard diluent, assay buffer, conjugate and substrate.

B_0 = 0 pmolg/mL standard; contains standard diluent, conjugate, antibody and substrate.

14. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Refer to the recommended plate layout in Section 12 before proceeding with the assay

14.1 If using samples prepared in 0.1M HCl, add 50 µL of Neutralizing Reagent into each well except the TA and Blank wells. Do not add Neutralizing Reagent for the other sample diluent options.

14.2 Add 100 µL appropriate diluent (Assay Buffer 2, 0.1 M HCl or non-conditioned culture media) into the NSB (non-specific binding).

14.3 Add 100 µL appropriate diluent (Assay Buffer 2,).1 M HCl or non-conditioned tissue culture media) into the B_0 (0 pmol/mL standard) wells.

14.4 Add 50 µL of standard diluent to NSB wells.

14.5 Add 100 µL of prepared standards and diluted samples to appropriate wells.

14.6 Add 50 µL of the Cyclic AMP Complete Alkaline Phosphatase (blue) into each well except the TA and Blank wells.

14.7 Add 50 µL of the Cyclic AMP Complete Antibody (yellow) into each well except the Blank, TA and NSB wells
Δ Note Every well used should be green in color except the NSB wells which should be blue. The Blank and TA wells are empty at this point and have no color.

14.8 Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided.

14.9 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

14.10 Add 5 µL of the Cyclic AMP Complete Alkaline Phosphatase (blue) to the TA wells.

14.11 Add 200 µL of the pNpp Substrate solution to every well.

14.12 Incubate at room temperature for 1.5 hours with shaking.

14.13 Add 50 µL Stop Solution into each well. The plate should be read immediately.

14.14 Blank the plate reader against the B_s wells, read the O.D. absorbance at 405 nm.

Assay Procedure Flow Chart:

Well ID	Blank A1, B1	TA C1, D1	NSB E1, F1	Zero Std. (Bo) G1, H1	Stds. A2 – B3	Samples C3 – H12
***Neutralizing Reagent**	---	---	50 µL	50 µL	50 µL	50 µL
Standard Diluent	---	---	150 µL	100 µL	---	---
Std. and/or Sample	---	---	---	---	100 µL	100 µL
Conjugate	---	---	50 µL	50 µL	50 µL	50 µL
Antibody	---	---	---	50 µL	50 µL	50 µL
Incubate 2 hours at RT, shaking	→→→	→→→	→→→	→→→	→→→	→→→
Asp. & Wash 3 x 200 µL	→→→	→→→	→→→	→→→	→→→	→→→
Conjugate	---	5 µL	---	---	---	---
Substrate	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL
Incubate 1.5 hours at RT, shaking	→→→	→→→	→→→	→→→	→→→	→→→
Stop Solution	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL

*** Neutralizing Reagent is only added if 0.1M HCl is used for preparation of standards and samples. Do not use with the other diluent options.**

15. Calculations

15.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

Average Net OD = Average OD - Average NSB OD

15.2 Plot the Average Net OD for each Standard versus the concentration of Cyclic AMP. The concentration of Cyclic AMP in the unknowns can be determined by interpolation of net OD values.

15.3 To normalise for protein content, divide the resulting picomole per mL determinations (pmol/mL) by the total protein concentration in each sample. This is expressed as pmol cAMP per mg total protein.

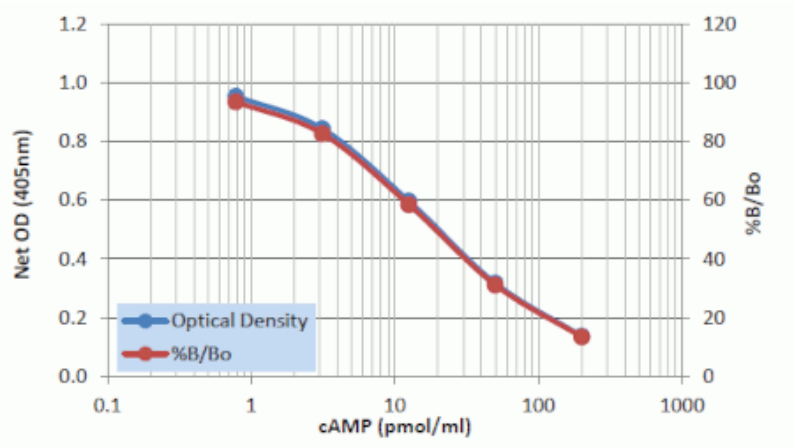
A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

16. Typical Data

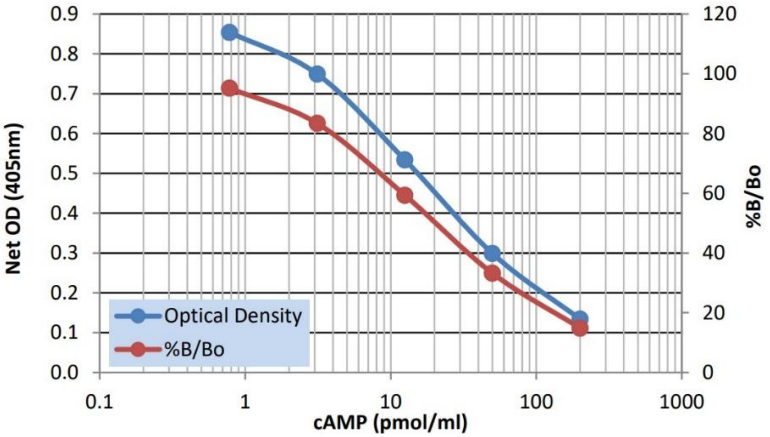
Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

Non-Acetylated Format in Assay Buffer 2:



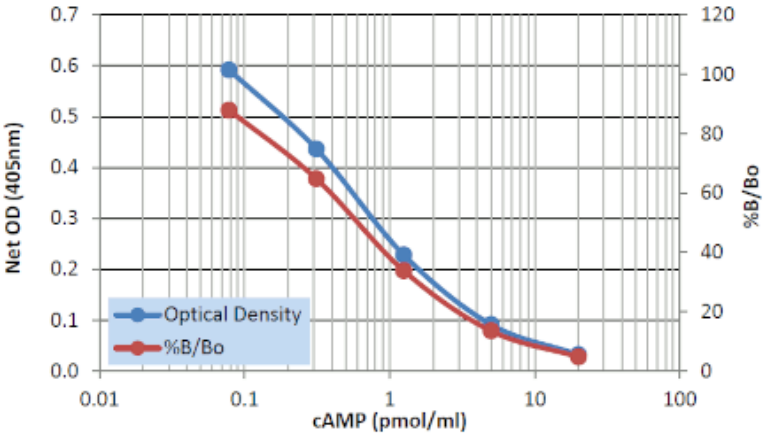
Sample	Mean OD	Net OD	%B/Bo	cAMP (pmol/mL)
NSB	0.102	-	-	-
Bo	1.123	1.021	-	0
S1	0.239	0.137	13.44	200
S2	0.42	0.318	31.13	50
S3	0.699	0.597	58.48	12.5
S4	0.945	0.843	82.55	3.125
S5	1.055	0.953	93.42	0.781

Non-Acetylated Format in 0.1M HCl:



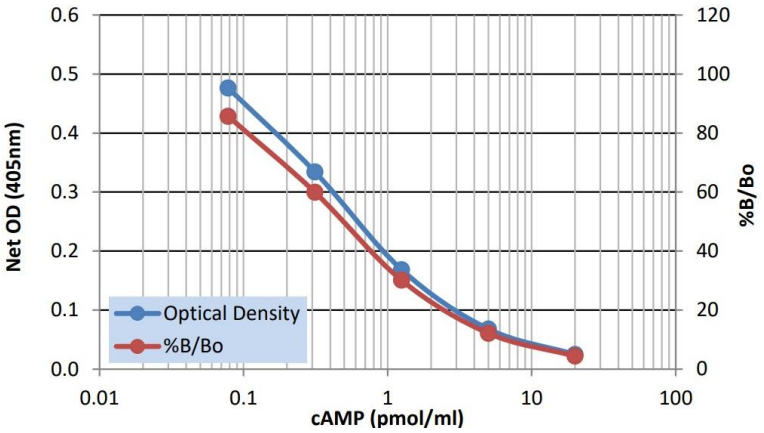
Sample	Mean OD	Net OD	%B/Bo	cAMP (pmol/mL)
NSB	0.104	-	-	-
Bo	1.004	0.900	-	0
S1	0.238	0.134	14.90	200
S2	0.403	0.299	33.22	50
S3	0.638	0.534	59.37	12.5
S4	0.853	0.749	83.39	3.125
S5	0.958	0.854	95.16	0.781

Acetylated Format in Assay Buffer 2:



Sample	Mean OD	Net OD	%B/Bo	cAMP (pmol/mL)
NSB	0.103	-	-	-
Bo	0.776	0.673	-	0
S1	0.135	0.032	4.74	20
S2	0.194	0.091	13.51	5
S3	0.331	0.228	33.83	1.25
S4	0.540	0.437	64.81	0.3125
S5	0.695	0.592	87.98	0.0781

Acetylated Format in 0.1M HCl:



Sample	Mean OD	Net OD	%B/Bo	cAMP (pmol/mL)
NSB	0.104	-	-	-
Bo	0.660	0.556	-	0
S1	0.129	0.025	4.45	20
S2	0.172	0.068	12.18	5
S3	0.273	0.169	30.12	1.25
S4	0.438	0.334	59.97	0.3125
S5	0.580	0.476	85.69	0.0781

17. Typical Sample Values

SENSITIVITY –

Assay Buffer 2

The sensitivity of the assay, defined as the concentration of cAMP measured at 2 standard deviations from the mean of 18 zeros along the standard curve, was determined to be 0.49 pmol/mL in the non-acetylated assay format and 0.027 pmol/mL in the acetylated assay format.

0.1M HCl

The sensitivity of the assay, defined as the concentration of cAMP measured at 2 standard deviations from the mean of 18 zeros along the standard curve, was determined to be 1.18 pmol/mL in the non-acetylated assay format and 0.006 pmol/mL in the acetylated assay format.

SAMPLE RECOVERY –

0.1M HCl should not be used to dilute culture supernatants, plasma, serum, or saliva samples.

Recovery was determined by Cyclic AMP standard being spiked into the following matrices diluted with Assay Buffer 2 and measured in the kit. Mean recoveries are as follows:

Non-Acetylated Format:

Sample Type	Average % Recovery	Minimum Recommended Dilution
EDTA plasma	127	1:16
Human Serum	133	1:64
Human Saliva	114	1:16

Acetylated Format:

Sample Type	Average % Recovery	Minimum Recommended Dilution
EDTA plasma	92.3	1:64
Human Serum	85	1:64
Human Saliva	130	1:2

LINEARITY OF DILUTION –

Plasma, saliva and serum samples containing cAMP were diluted to their respective minimum recommended dilutions (MRD) and serially diluted 1:2 in Assay Buffer. The results are as follows:

Non-Acetylated:

Dilution	EDTA plasma (%)	Saliva (%)	Serum (%)
1:16	100	100	-
1:32	104	92	-
1:64	111	88	100
1:128	103	90	99
1:256	104	85	108
1:512	103	80	110
1:1024	104	86	120
1:2048	113	139	125
1:4096	-	-	145
1:8192	-	-	162

Acetylated:

Dilution	EDTA plasma (%)	Saliva (no cAMP) (%)	Serum (%)
Neat	-	-	-
1:2	-	100	-
1:4	-	75	-
1:8	-	63	-
1:16	-	83	-
1:32	-	92	-
1:64	100	144	100
1:128	110	194	96
1:256	136	-	140
1:512	172	-	164
1:1024	122	-	138
1:2048	122	-	153
1:4096	167	-	139

Cell and tissue lysate samples containing cAMP was serially diluted 1:2 in the 0.1M HCl diluent and measured in the assay. The results are shown in the tables below.

Non-Acetylated:

Dilution	Cell lysate (%)	Tissue lysate (%)	Tissue lysate, treated (%)
1:4	100	100	100
1:8	100	84	110
1:16	139	123	120
1:32	148	125	121
1:64	134	122	110
1:128	151	131	104
1:256	139	138	87
1:512	134	-	82

Acetylated:

Dilution	Cell lysate (%)	Tissue lysate (%)	Tissue lysate, treated (%)
1:32	100	100	100
1:64	99	121	145
1:128	177	189	196
1:256	145	188	175
1:512	97	146	168
1:1024	139	186	170
1:2048	158	199	183
1:4096	175	179	207

PRECISION –

Intra-assay was determined by assaying 20 replicates of three buffer controls containing cAMP in a single assay.

Inter-assay was determined by measuring buffer controls of varying cAMP concentrations in multiple assays over several days.

Non-Acetylated Format (Assay Buffer 2):

Intra-Assay

pmol/mL	%CV
20	4.19
5	11.94
2	13.38

Inter Assay

pmol/mL	%CV
20	8.1
5	10.41
2	10.99

Acetylated Format (Assay Buffer 2):

Intra-Assay

pmol/mL	%CV
2	4.32
0.5	5.42
0.2	5.91

Inter-Assay

pmol/mL	%CV
2	8.94
0.5	10.77
0.2	12.18

Non-Acetylated Format (0.1M HCL):

Intra-Assay

pmol/mL	%CV
20	3.08
5	11.59
2	6.13

Inter-Assay

pmol/mL	%CV
20	10.68
5	11.94
2	13.95

Acetylated Format (0.1M HCL):

Intra-Assay

pmol/mL	%CV
2.0	4.09
0.5	4.41
0.2	6.14

Inter-Assay

pmol/mL	%CV
2.0	18.69
0.5	13.29
0.2	12.92

18. Assay Specificity

CROSS REACTIVITY –

The cross reactivities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration of ten times the high standard. These samples were then measured in the assay:

Cyclic AMP	100 %
AMP	<0.001 %
ATP	<0.001 %
cGMP	<0.001 %
GMP	<0.001 %
GTP	<0.001 %
cUMP	<0.001 %
CTP	<0.001 %

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

20. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)