Cyclic AMP Competitive ELISA Kit

For lysate and homogenate samples

Catalog Number: EMSCAMPL

Product description

A competitive immunoassay for the quantitative determination of cyclic AMP (cAMP) in samples treated with 0.1 M HCl. The assay is based on the competition between cAMP in the standard or sample and Alkaline Phosphatase conjugated cAMP (cAMP-AP) for a limited amount of cAMP monoclonal antibody bound to an Anti-Rabbit IgG precoated 96-well plate. As the concentration of cAMP in the sample increases, the amount of cAMP-AP captured by the coating antibody decreases. Thus, there is an inverse relationship between optical density (OD) and the amount of analyte in the sample.

Contents and storage

Upon receipt, store the kit at –20°C.

Description	Size
Antibody coated 96-well plate	1 plate
cAMP Antibody	5 mL
cAMP-AP Conjugate	5 mL
0.1 M HCl	27 mL
Neutralizing Reagent	5 mL
Triethylamine*	2 mL
Acetic Anhydride*	1 mL
cAMP Standard (2,000 pmol/mL)	0.5 mL
20X Wash Buffer	27 mL
pNpp Substrate Solution	20 mL
Stop Solution	5 mL
Plate Sealer	1 each

CAUTION! * Some components are corrosive, flammable, or produce harmful vapor. Keep away from open flames, hot surfaces, and sources of ignition. Use only under a chemical fume hood.

Additional required materials

- Deionized or distilled water
- Precision pipettes (for volumes between 5 µL and 1,000 µL)
- Repeater pipettes (for dispensing 50 µL and 200 µL)
- Disposable beaker for diluting buffer concentrates
- 12 × 75 mm glass tubes
- Graduated cylinders
- Microplate shaker
- Absorbent lint free paper for blotting
- Microplate reader capable of reading at 405 nm, preferable with correction between 570 and 590 nm
- Triton™ X-100 (optional for sample preparation)
- Liquid nitrogen, mortar & pestle, and concentrated HCl (optional - for tissue samples)

General guidelines

- Do not mix components from different kit lots or use reagents beyond the kit expiration date.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase (AP). Contaminating AP activity, especially in the substrate solution, may lead to high blanks.
- Care should be taken not to touch pipette tips and other items that are used in the assay with bare hands.
- Allow kit components to come to room temperature for at least 30 minutes before use.
- 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.

Assay compatibility

The cAMP Competitive ELISA for lysate and homogenate samples is compatible with cAMP samples that have been treated with hydrochloric acid to stop endogenous phosphodiesterase activity. Samples containing rabbit IgG will interfere with the assay.

Prepare 1X Wash Buffer

1. Allow the 20X Wash Buffer to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 5 mL of 20X Wash Buffer with 95 mL of deionized water. Label as 1X Wash Buffer.

   The diluted buffer is stable for up to 3 months at room temperature.

Prepare tissue homogenate

1. Grind tissue samples frozen in liquid nitrogen to a fine powder under liquid nitrogen in a stainless steel mortar.
2. Allow the liquid nitrogen to evaporate, then weigh the frozen tissue and homogenize in 10 volumes of 0.1 M HCl.
3. Centrifuge at ≥600 × *g* for 10 minutes to pellet debris.
4. The sample may be further diluted in the 0.1 M HCl provided and run directly in the assay or stored frozen for later analysis.

Prepare cell lysates

1. Remove tissue culture media from cells by aspiration or centrifugation and treat with 0.1 M HCl (~1 x 10^6 cells per ml).

   Note: Cell lysis can be enhanced by adding 0.1- 1% Triton™ X-100 to the 0.1 M HCl prior to use. The detergent does not interfere with acetylation or the binding portion of the assay in this concentration range, but a modest increase in the optical density will be observed.
2. Incubate for 10 minutes at room temperature and verify cell lysis by visual inspection. Incubate for an additional 10 minutes if adequate lysis has not occurred.
3. Centrifuge at ≥600 × *g* at room temperature for 10 minutes.
4. Use the supernatant directly in the assay or store frozen for later analysis.

Sample preparation guidelines

- Samples in matrix treated with hydrochloric acid can be measured directly without evaporation or further treatment.
- Acetylate samples and standards for samples with very low levels of cAMP.
- To perform the **acetylated version** of the assay, acetylate samples by adding 10 µL of the Acetylating Reagent for every 200 µL of sample.
- Use **acetylated samples** within 30 minutes.

Prepare Acetylating Reagent

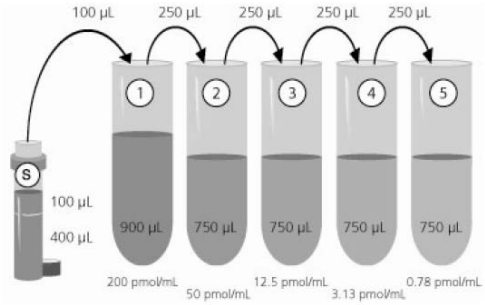
- Add 0.5 mL of acetic anhydride to 1 mL triethylamine and mix well.
- Use reagent within 60 minutes of preparation.

Standard preparation guidelines

- If using samples that are acetylated, or treated with Triton™ X-100, ensure the standard curve is prepared in the same type of matrix.
- Allow the stock Standard to warm to room temperature before use.
- Label five glass 12 x 75 mm tubes #1 through #5 to be used for standard preparation.

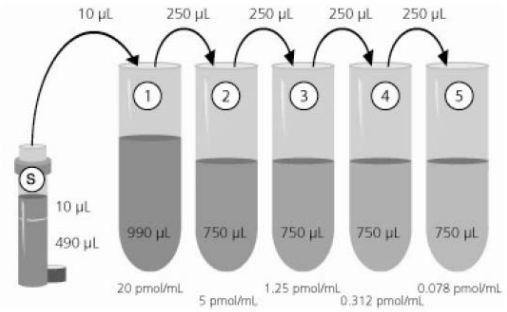
Dilute non-acetylated standards

1. Add 900 µL 0.1 M HCl to Tube #1.
2. Add 750 µL 0.1 M HCl to Tubes #2 to #5.
3. Add 100 µL cAMP Standard to Tube #1 and vortex thoroughly
4. Add 250 µL of Tube #1 to Tube #2 and vortex thoroughly.
5. Continue to prepare serial dilutions of the standard as shown in the diagram below.
   Use the non-acetylated standards within 60 minutes.



Dilute acetylated standards

1. Add 990 µL 0.1 M HCl to Tube #1.
2. Add 750 µL 0.1 M HCl to Tubes #2 to #5.
3. Add 10 µL cAMP Standard to Tube #1.
4. Make serial dilutions of the standard as described below in the dilution diagram.



5. For each tube, add 10 µL of the Acetylating Reagent for every 200 µL of standard and vortex immediately.
6. To an additional tube add 1 mL of 0.1 M HCl and 50 µL of Acetylating Reagent and label as Zero Standard/NSB .
7. Use the acetylated standards within 30 minutes.

ELISA procedure

Allow all reagents to warm to room temperature for at least 30 minutes before use. Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

Run all standards and samples in duplicate.

1. Add 50 µL of Neutralizing Reagent into each well, **except** the Total Activity (TA) and Blank wells.
2. Add 100 µL of 0.1 M HCl to NSB and B_0 (0 pmol/mL Standard) wells.
3. Add 50 µL of 0.1 M HCl to NSB wells.
4. Add 100 µL of Standards #1 through #5 into the appropriate wells.
5. Add 100 µL of the Samples into the appropriate wells.
6. Add 50 µL of the blue cAMP-AP Conjugate into each well, **except** the TA and Blank wells.
7. Add 50 µL of the yellow cAMP Antibody into each well, **except** the TA, Blank, and NSB wells.

   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.
8. Seal the plate and incubate at room temperature on a plate shaker (~500 rpm) for 2 hours.
9. Empty the contents of the wells and wash by adding 400 µL of the 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes.
10. 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.
11. Add 5 µL of the blue cAMP-AP Conjugate to the TA wells.
12. Add 200 µL of pNpp Substrate Solution to each well, and incubate at room temperature for 1 hour (no shaking).
13. Add 50 µL of Stop Solution to every well and read the plate immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculations

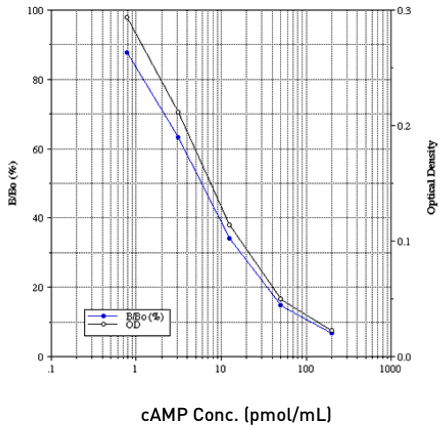
Several options are available for the calculation of the concentration of cAMP in the samples. It is recommended that the data be analyzed by a 4 parameter logistic curve-fitting program.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

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

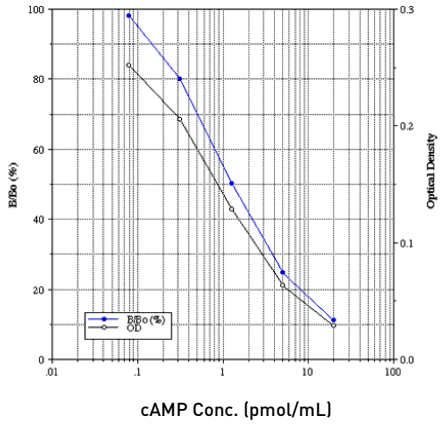
Typical standard curve

A typical standard curve for the **non-acetylated version** of the assay is shown below. This curve must not be used to calculate cAMP concentrations; a standard curve must be run with every assay.



Typical standard curve

A typical standard curve for the **acetylated version** of the assay is shown below. This curve must not be used to calculate cAMP concentrations; a standard curve must be run with every assay.



Performance characteristics

Sensitivity

The minimum detectable dose of cAMP is listed in the following table.

Assay	Sensitivity
Non-acetylated version	0.39 pmol/mL
Acetylated version	0.037 pmol/mL

Sensitivity was defined as the concentration of cAMP measured at 2 standard deviations from the mean of 16 zeros along the standard curve.

Linearity

0.1M HCl sample containing cAMP was serially diluted 1:2 in the 0.1M HCl diluent and measured in the assay. The results are shown in the table below.

Non-acetylated			
Dilution	**Expected (pmol/mL)**	**Observed (pmol/mL)**	**Recovery (%)**
Neat	**—**	**15.44**	**—**
1:2	**7.72**	**8.24**	**107%**
1:4	3.86	3.67	95%
1:8	1.93	2.32	120%

Acetylated			
Dilution	**Expected (pmol/mL)**	**Observed (pmol/mL)**	**Recovery (%)**
Neat	**—**	**3.41**	**—**
1:2	**1.70**	**2.03**	**119%**
1:4	0.85	0.95	111%
1:8	0.43	0.49	115%

Sample recovery

cAMP concentrations were measured in tissue culture media. cAMP was spiked into the undiluted sample which was diluted with the kit 0.1 M HCl and then assayed in the kit.

Sample	Non-acetylated		Acetylated	
	% Recovery	Dilution	% Recovery	Dilution
Tissue Culture Media	94.8%	1:4	95.2%	1:4

0.1 M HCl should not be used to dilute culture supernatants (without pre-treatment with concentrated HCl), serum, or saliva samples..

Precision

Intra-assay precision was determined by assaying 20 replicates of three 0.1M HCl controls containing cAMP in a single assay.

Intra-assay	Non-acetylated		Acetylated	
	cAMP (pmol/mL)	%CV	cAMP (pmol/mL)	%CV
Low	1.24	8.9	0.679	4.6
Medium	6.31	4.3	3.58	8.4
High	35.92	8.3	NT	NT

Inter-assay precision was determined by measuring 0.1M HCl controls of varying cAMP concentrations in multiple assays over several days.

Inter-assay	Non-acetylated		Acetylated	
	cAMP (pmol/mL)	%CV	cAMP (pmol/mL)	%CV
Low	1.18	13.1	1.29	13.6
Medium	5.53	4.2	5.62	7.8
High	30.36	11.6	NT	NT

Specificity

The cross-reactivities for a number of related compounds were determined by dissolving the cross reactant in 0.1 M HCl at concentrations from 500,000 to 500 pmol/mL. These samples were then measured in the cAMP assay and the measured cAMP concentration at 50% B/B_0 calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
cAMP	100%
AMP	0.33%
ATP	0.12%
cGMP, GMP, GTP, cUMP, CTP	<0.001%

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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