Datasheet for ABIN577668

CAMP CLIA Kit

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Overview

Quantity:	96 tests
Target:	CAMP (cAMP)
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Minimum Detection Limit:	< 1 fM cAMP/Sample
Application:	ELISA

Product Details

Purpose:	The DetectX® Direct High Sensitivity Cyclic AMP (cAMP) Chemiluminescent Immunoassay kit is designed to quantitatively measure cAMP present in lysed cells, EDTA and heparin plasma, urine, saliva and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Cell Lysate, Saliva, Urine, Plasma (EDTA), Plasma (heparin), Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Chemiluminescent
Specificity:	Species Independent. Samples Types validated: Cell Lysates, Saliva, Urine, EDTA and Heparin Plasma, Tissue Culture Media
Cross-Reactivity (Details):	(%) Cyclic AMP 100 % AMP < 0.08 % GMP < 0.08 % Cyclic GMP < 0.08 % ATP < 0.08 %
Sensitivity:	0.119 pmol/mL
Characteristics:	The Cyclic AMP (cAMP) Chemiluminescent Direct Immunoassay (CLIA) kits are designed to

	quantitatively measure cAMP present in cell lysates, plasma, urine, saliva, tissue and culture media samples. The supplied Sample Diluent will lyse cells, stabilize cAMP and stop phosphodiesterase activity. A cAMP standard is provided to generate a standard curve for the assay. The supplied Plate Primer solution is added to the wells of a coated white microtiter plate, followed by standards or diluted samples. A cAMP-peroxidase conjugate is then added to the wells. The binding reaction is initiated by the addition of a polyclonal antibody to cAMP. After a 2 hour incubation the plate is washed and chemiluminescent substrate is added. The substrate immediately reacts with the bound cAMP-peroxidase conjugate. The generated chemiluminescent glow signal is measured. The concentration of the cAMP in the sample is calculated, after making correction for the dilution. Cyclic AMP (cAMP) is one of the most important second messengers and a key intracellular regulator. Discovered by Sutherland and Rall in 1957, it functions as a mediator of activity for a number of hormones, including epinephrine, glucagon, and ACTH. Cyclic AMP is produced by the enzyme adenylate cyclase, and the enzyme is activated by the hormones glucagon and adrenaline and by G protein. cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase. Other biological actions of cAMP include regulation of innate immune functioning, axon regeneration, cancer, and inflammation.
Components:	Coated White 96 Well Plates White plastic microtiter plate(s) coated with donkey anti-sheep IgG. 1 Or 5 Each Cyclic AMP Standard 125 µL Cyclic AMP at 1,500 pmol/mL in a special stabilizing solution. DetectX® Cyclic AMP Antibody A sheep antibody specific for cyclic AMP. 3 mL Or 13 mL DetectX® Cyclic AMP Conjugate Concentrate A 50X cyclic AMP-peroxidase conjugate concentrate stock in a special stabilizing solution. 60 µL Or 260 µL Conjugate Diluent Contains special stabilizers and additives. 3 mL Or 13 mL Sample Diluent Concentrate Now Supplied ONLY as Concentrate Contains special stabilizers and additives. The 4X concentrate must be diluted with deionized or distilled water. CAUSTIC 12 mL Or 60 mL Plate Primer A neutralizing solution containing special stabilizers and additivies. 25 mL Acetic Anhydride 2mL Acetic Anhydride WARNING: Corrosive Lachrymator Triethylamine 4mL Triethylamine WARNING: Corrosive Lachrymator Wash Buffer Concentrate A 20X concentrate that must be diluted with deionized or distilled water. 30 mL Or 125 mL Substrate Solution A 6mL Or 28 mL Substrate Solution B 6mL Or 28 mL Plate Sealer 1 Or 5 Each

Product Details

Material not included:	Distilled or deionized water. Repeater pipet and disposable tips capable of delivering 25 and 100 µL. Microplate shaker. 96 well microplate reader capable of reading glow chemiluminescence. A list of some models of suitable readers can be found on our website at www.ArborAssays.com/resources/lit.asp. All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol: Dilute 5 µL of the Cyclic AMP Conjugate Concentrate into 995 µL of deionized water. Pipet 5 µL of diluted conjugate into 245 µL of deionized water. Pipet 5 µL of this mixture into a white well and add 100 µL of prepared CLIA substrate (see page 9 for details). This well will give you an intensity slightly above the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the readers maximum signal. To properly analyze the data software will be required for converting raw RLU readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Target Details

Target:	CAMP (cAMP)
Alternative Name:	Cyclic AMP (cAMP Products)
Target Type:	Chemical
Background:	Adenosine-3',5'-cyclic monophosphate, or cyclic AMP (cAMP), C10H12N5O6P, is one of the most im- portant second messengers and a key intracellular regulator. Discovered by Sutherland and Rall in 19571, it functions as a mediator of activity for a number of hormones, including epinephrine, glucagon, and ACTH2-4. Adenylate cyclase is activated by the hormones glucagon and adrenaline and by G protein. Liver adenylate cyclase responds more strongly to glucagon, and muscle adeny- late cyclase responds more strongly to adrenaline. cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase. In the Human Metabolome Database there are 166 metabolic enzymes listed that convert cAMP5. Other biological actions of cAMP include regulation of innate immune functioning6, axon regeneration7, cancer8, and inflammation9
Pathways:	Cellular Response to Molecule of Bacterial Origin

Application Details

Application Notes:	This assay has been validated for lysed cells, saliva, urine, EDTA and heparin plasma samples and for tissue culture media samples. Samples should be stored at -70 °C for long term storage. 24-Hour urine samples may need to have 1 mL concentrated hydrochloric acid added for every 100 mL volume to act as a preservative. Samples containing visible particulate should be centrifuged prior to using. Cyclic AMP is identical across all species and we expect this kit may measure cAMP from sources other than human. The end user should evaluate recoveries of cAMP in other samples being tested. After dilution in the Sample Diluent (see page 9) there may be some precipitation of proteins. This precipitate will not effect the results obtained. After being diluted in Sample Diluent the samples can be assayed directly within 2 hours, or frozen at ≤ -70 °C for later analysis. Severely hemolyzed samples should not be used in this kit. For samples containing low levels of cAMP and for all plasma samples, the acetylated assay protocol must be used due to its enhanced sensitivity. All standards and samples should be diluted in glass test tubes.
Comment:	Sample values: Fourteen human plasma samples were tested in the assay. Diluted samples were acetylated and run in the Acetylated Format. Values ranged from 12.5 to 43.32 pmol/mL with an aver- age for the samples of 21.15 pmol/mL. The normal reference range for cAMP in plasma is 3.9-13.7 pmol/mL10. Four human urine samples were diluted > 1:30 in Sample Diluent and values ranged in the neat samples from 1,099 to 4,585 pmol/mL with an average for the samples of 3,034 pmol/ mL. The normal reference range for cAMP in urine is 800-12,000 pmol/mL11. Two human saliva samples were diluted 1:4 in Sample Diluent and run in both the Regular and Acetylated Formats. Values ranged from 5.8 to 6.6 pmol/mL with an average of 6.2 pmol/mL in the neat samples. The normal range for cAMP in saliva is 3.4-17.2 pmol/mL12.
Assay Time:	2 h
Plate:	Pre-coated
Protocol:	For tissue samples, saliva and urine, where the levels of cAMP are expected to be relatively high, the regular format for the assay can be used. For plasma samples and some dilute cell lysates an optional acetylation protocol can be used.

	This kit can measure as little as 1 femtomol cAMP per sample. The kit is unique in that all samples and standards are diluted into an acidic Sample Diluent, which contains special additives and stabilizers, for cAMP measurement. This allows plasma, urine and saliva samples to be read in an identical manner to lysed cells. Acidified samples of cAMP are sta- ble and endogenous phosphodiesterases are inactivated in the Sample Diluent. A cAMP standard is provided to generate a standard curve for the assay and all samples should be read off the stan- dard curve. A white microtiter plate coated with an antibody to capture sheep IgG is provided. Prior to the addition of any samples or standards a neutralizing Plate Primer solution is added to all the used wells. Standards or diluted samples, either with or without acetylation, are pipetted into the primed wells. A cAMP-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a sheep antibody to cAMP to each well. After a 2 hour incubation, the plate is washed and the chemiluminescent substrate is added. The substrate reacts with the bound cAMP-peroxidase conjugate to produce light The generated light is detected in a microtiter plate reader capable of reading luminescence. The concentration of the cAMP in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30-60 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine cAMP concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Wash Buffer Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months. Sample Diluent Now Supplied ONLY as Concentrate Prepare the Sample Diluent by diluting the Sample Diluent Concentrate 1:4, adding one part of the concentrate to three parts of deionized water. Once diluted this is stable at 4 °C for 3 months. Cyclic AMP Conjugate The supplied Cyclic AMP Conjugate Concentrate should be diluted 1:50 with the Conjugate Diluent as indicated in the table below. Once diluted the Cyclic AMP conjugate is stable for one month when stored at 4 °C. 1 Plate 2

Plates 3 Plates 4 Plates 5 Plates Conjugate Concentrate 50 µL 100 µL 150 µL 200 µL 250 µL Conjugate Diluent 2.45 mL 4.9 mL 7.35 mL 9.8 mL 12.25 mL Final Mixture 2.5 mL 5 mL 7.5 mL 10 mL 12.5 mL Chemiluminescent Substrate Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle.

Once mixed the substrate is stable for one month when stored at 4 °C. ® www.ArborAssays.com 9 WEB INSERT 150617 reagent preparatiOn - regular fOrmat Use this format for urine, saliva and some cell lysates.

Do NOT use for plasma samples.

All standards and samples should be diluted in glass test tubes.

Standard Preparation - regular fOrmat Label one glass test tube as Stock 2 and five tubes as #1 through #5.

Pipet 90 µL of Sample Di- luent into the Stock 2 tube and 450 µL of Sample Diluent into tube #1.

Pipet 300 µL of Sample Diluent into tubes #2 to #5.

The Cyclic AMP stock solution contains an organic solvent.

Prerinse the pipet tip several times to ensure accurate delivery.

Carefully add 10 µL of the cAMP stock solution to the Stock 2 tube and vortex completely.

Take 50 µL of the cAMP solution in the Stock 2 tube and add it to tube #1 and vortex completely.

Take 150 µL of the cAMP solution in tube #1 and add it to tube #2 and vortex completely.

Repeat the serial dilutions for tubes #3 through #5.

The concentration of Cyclic AMP in tubes 1 through 5 will be 15, 5, 1.667, 0.556, and 0.185 pmol/mL.

Non-Acetylated Stock 2 Std 1 Std 2 Std 3 Std 4 Std 5 Sample Diluent (µL) 90 450 300 300 300 300 Addition Cyclic AMP Std.

Stock 2 Std 1 Std 2 Std 3 Std 4 Vol of Addition (µL) 10 50 150 150 150 150 Final Conc (pM/ mL) 150 15 5 1.667 0.556 0.185 Use Standards within 1 hour of preparation. ® www.ArborAssays.com 10 WEB INSERT 150617 aSSay prOtOcOl - regular fOrmat 1.

Use the plate layout sheet on the back page to aid in proper sample and standard identification.

Determine the number of wells to be used and return unused wells to the foil pouch with desiccant.

Seal the ziploc plate bag and store at 4°C. 2.

Add 50 µL of Plate Primer into all wells used. failure tO add plate primer tO all WellS firSt Will cauSe aSSay tO fail. 3.

Pipet 75 µL Sample Diluent into the non-specific binding (NSB) wells. 4.

	Pipet 50 µL of Sample Diluent into wells to act as maximum binding wells (B0 or 0 pg/ mL). 5. Pipet 50 µL of samples or standards into wells in the plate. NOTE: Sample Diluent will turn from orange to bright pink upon sample or standard addition to the Plate Primer in the wells. 6. Add 25 µL of the diluted DetectX® cAMP Conjugate to each well using a repeater pipet. 7. Add 25 µL of the DetectX® cAMP Antibody to each well, except the NSB wells, using a repeater pipet. 8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken, signals bound will be approximately 25 % lower. 9. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels. 10. Add 100 µL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet. 11. Incubate the plate at room temperature for 5 minutes without shaking. 12. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40 % over 60 minutes. 13. Use the plate reader's built-in 4PLC software capabilities to calculate cAMP concentration for each sample.
Sample Preparation:	Cells Cell lysis buffers containing high concentrations of SDS or other detergents may not be compat- ible with this assay or may require extra dilution. Please read Interferents section on page 22 for more information. This kit is compatible with either adherent or non-adherent cells. The cells can be grown in any suitable sterile containers such as Petri dishes, 12-, 48- or 96-well culture plates or flasks. The cells must be isolated from the media prior to being lysed with the provided Sample Diluent. The acidic Sample Diluent contains detergents to lyse the cells, inactivate endogenous phosphodiesterases and stabilize the cAMP. Some cell types are extremely hardy and the end user should optimize the lysis conditions utilizing freeze-thaw cycles and ultrasonic treatments to fully lyse their cells. We used ~ 107 Jurkat cells per mL of Sample Diluent. Cell number needs to be determined by the end user since it will be dependant on cell type and treatment conditions. Care must be taken not to over dilute the samples. For adherent cells, the media should be aspirated from the cells and the cells washed with PBS. The adherent cells should be treated directly with the Sample Diluent for 10 minutes at room temperature. Cells can be scraped to dislodge them from the plate surface and cells should be inspected to ensure lysis. Detergent has been added to the Sample Diluent to help lysis occur. Centrifuge the samples at ≥ 600 x g at 4 °C for 15 minutes and assay the supernatant directly. If

	required, the TCM can be assayed for cAMP as outlined below. For non-adherent cells, pellet and wash the cells with PBS by centrifuging the samples at ≥ 600 x g at 4 °C for 15 minutes as described above. Treat the aspirated, washed pellet directly with the Sample Diluent for 10 minutes at room temperature. Cells should be inspected to ensure lysis. Detergent has been added to the Sample Diluent to help lysis occur. Centrifuge the samples at ≥ 600 x g at 4 °C for 15 minutes and assay the supernatant directly. If required, the TCM can be assayed for cAMP as outlined below.
Calculation of Results:	All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. Average the duplicate RLU readings for each standard and sample. Cre- ate a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean RLU's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values. Or use the online tool from http://www.myassays.com/arbor-assays-cyclic-amp-direct-chemiluminescent-eia-kit-non-acetyl.assay to calculate the data. *The MyAssays logo is a registered trademark of MyAssays Ltd. typical data - regular fOrmat Sample Mean RLU Net RLU % B/B0 Cyclic AMP Conc. (pmol/mL) NSB 19,240 0 - - Standard 1 30,850 11,610 10.7 15 Standard 2 51,385 32,145 29.6 5 Standard 3 77,065 57,825 53.3 1.667 Standard 4 102,120 82,880 76.4 0.556 Standard 5 120,730 101,490 93.6 0.185 B0 127,670 108,430 100.0 0 Sample 1 42,405 23,165 21.4 7.71 Sample 2 71,190 51,950 47.9 2.14 Always run your own standard curve for calculation of results. Do not use this data ® www.ArborAssays.com 12 WEB INSERT 150617 Typical Standard Curve - Regular Format ' & % & $ # $ 0 0 0 0 1]\Q ^[]Z [: Always run your own standard curve for calculation of results. Do not use this data. ValidatiOn data - regular fOrmat Sensitivity and Limit of Detection Sensitivity was calculated by comparing the RLU's for eighteen wells run for each of the B0 and standard #5. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. Sensitivity was determined as 0.119 pmol/mL. This is equivalent to 5.95 fmol cAMP per well. The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty runs for each of the zero standard and a low concentration human urine sample.

Application Details

	Limit of Detection was determined as 0.076 pmol/mL. This is equivalent to 3.8 fmol cAMP per well. ® www.ArborAssays.com 13 0 0 Prior to running the acetylated assay, all standards, samples and the Sample Diluent used for the B0 and NSB wells must be acetylated. Acetylation is carried out by adding 15 µL of the Acetyla- tion Reagent (as prepared below) for each 300 µL of the standard, sample and Sample Diluent. After addition of the Acetylation Reagent immediately vortex each treated standard, sample or Sample Diluent and use within 30 minutes of preparation. Note: Upon Acetylation, all of the standards and samples diluted in the orange Sample Diluent will change to a pale yellow colour. reagent preparatiOn - acetylated fOrmat Acetylation Reagent Working in a fume hood mix one part of Acetic Anhydride with 2 parts of Triethylamine in a glass test tube. Use the following table to help determine the amount of Acetylation Reagent to make. Reagents Number of Samples to be Tested 20 40 100 200 Acetic Anhydride Volume (µL) 200 400 1,000 2,000 Triethylamine Volume (µL) 400 800 2,000 4,000 Acetylation Reagent Vol (mL) 0.6 1.2 3 6 Use the Acetylation Reagent within 60 minutes of preparation.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had laborato- ry safety instruction. The complete insert should be read and understood before attempting to use the product. This kit utilizes a peroxidase-based readout system. Buffers, including other manufac- turers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 9. The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly. The supplied Sample Diluent and Sample Diluent Concentrate are acidic. Take appropriate precau- tions when handling these reagents. The kit uses acetic anhydride and triethylamine as acetylation reagents. Triethylamine and acetic anhydride are lachrymators.

Handling

	Caution - corrosive, flammable, and harmful vapor. Use in hood with proper ventilation and wear appropriate protective safety wear.
Storage:	4 °C
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Publications

Product cited in:

Suffredini, Li, Xu, Moayeri, Leppla, Fitz, Cui, Eichacker: "Shock and Lethality with Anthrax Edema Toxin in Rats are Associated with Reduced Arterial Responsiveness to Phenylephrine and are Reversed with Adefovir." in: **American journal of physiology. Heart and circulatory physiology**, pp. ajpheart.00285.2017, (2017) (PubMed).

Inoue, Shimizu, Masui, Tsubonoya, Hayakawa, Sudoh: "Agarwood Inhibits Histamine Release from Rat Mast Cells and Reduces Scratching Behavior in Mice: Effect of Agarwood on Histamine Release and Scratching Behavior." in: **Journal of pharmacopuncture**, Vol. 19, Issue 3, pp. 239-245, (2016) (PubMed).

Yulia, Singh, Lei, Sooranna, Johnson: "Cyclic AMP Effectors Regulate Myometrial Oxytocin Receptor Expression." in: **Endocrinology**, Vol. 157, Issue 11, pp. 4411-4422, (2016) (PubMed).

McKinney, Eum, Dhara, Strand, Brown: "Calcium influx enhances neuropeptide activation of ecdysteroid hormone production by mosquito ovaries." in: **Insect biochemistry and molecular biology**, Vol. 70, pp. 160-9, (2016) (PubMed).

Fleckenstein, Rasko: "Overcoming Enterotoxigenic Escherichia coli Pathogen Diversity: Translational Molecular Approaches to Inform Vaccine Design." in: **Methods in molecular biology (Clifton, N.J.)**, Vol. 1403, pp. 363-83, (2016) (PubMed).

There are more publications referencing this product on: Product page

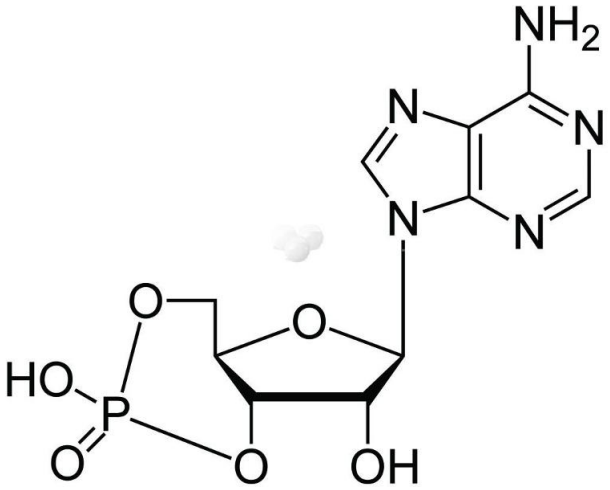

Image 1.

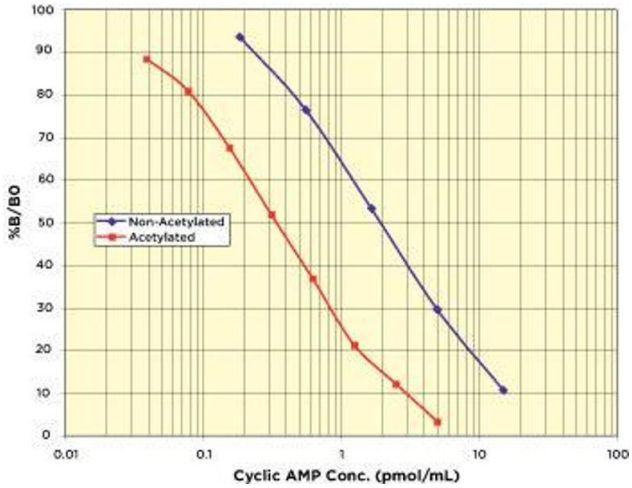

Image 2.

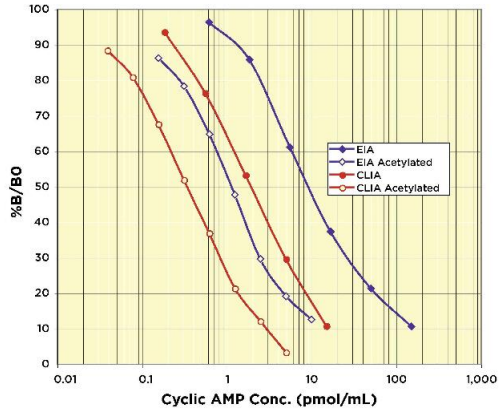

Image 3.

Please check the product details page for more images. Overall 4 images are available for ABIN577668.