Datasheet for ABIN577670

CAMP ELISA Kit

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Overview

Quantity:	96 tests
Target:	CAMP (cAMP)
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Minimum Detection Limit:	< 5 fM cAMP/Sample
Application:	ELISA

Product Details

Purpose:	The DetectX® Direct Cyclic AMP (cAMP) Immunoassay kit is designed to quantitatively measurecAMP present in lysed cells, EDTA and heparin plasma, urine, saliva and tissue culture mediasamples.
Brand:	DetectX®
Sample Type:	Cell Lysate, Saliva, Urine, Plasma (EDTA), Plasma (heparin), Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
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:	0.64 pmol/mL
Characteristics:	The Cyclic AMP (cAMP) Direct Immunoassay kit is designed to quantitatively measure cAMP

	present in lysed cells, tissue, plasma, urine, saliva and culture media samples. The kit is unique in that all samples and standards are diluted into an acidic Sample Diluent which contains cAMP stabilizers and is designed to lyse cells. A cAMP standard is provided to generate a standard curve for the assay. A special Plate Primer is added to all the wells and standards or samples in Sample Diluent are pipetted into the primed clear microtiter plate. A cAMP-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of an antibody to cAMP. After 2 hours, the plate is washed and substrate is added. The substrate reacts with the bound cAMP-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is read. An optional Acetylation Format allows super low concentrations of cAMP to be measured. Adenosine-3',5'-cyclic monophosphate, or 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. Adenylate cyclase is activated by the hormones glucagon and adrenaline and by G protein. Liver adenylate cyclase responds more strongly to glucagon, and muscle adenylate 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 cAMP.
Components:	Coated Clear 96 Well plates A clear plastic microtiter plate(s) coated with donkey anti-sheep IgG. 1 or 5 Each Cyclic Amp Standard Cyclic AMP at 1,500 pmol/mL in a special stabilizing solution. 125 or 625 µL DetectX® Cyclic Amp Antibody A sheep antibody specific for cyclic AMP. 3 mL or 13 mL DetectX® Cyclic Amp Conjugate A cyclic AMP-peroxidase conjugate in a special stabilizing solution. 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 WARninG: Corrosive lachrymator 2mL triethylamine WARninG: Corrosive lachrymator 4mL Wash Buffer Concentrate A 20X concentrate that must be diluted with deionized or distilled water. 30 mL or 125 mL TMB Substrate 11 mL or 55 mL Stop Solution A 1M solution of hydrochloric acid. CAUSTIC. 5 mL or 25 mL

Product Details

	Plate Sealer 1 or 5 Each
Material not included:	Distilled or deionized water. Repeater pipet with disposable tips capable of dispensing 25 µL, 50 µL and 100 µL. A microplate shaker. Colorimetric 96 well microplate reader capable of reading optical density at 450 nm. Software for converting raw relative optical density 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), C H N O P, is one of the most 10 12 5 6 important 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 adenylate 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 and the supernatant from the centrifuged samples used. 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: Seven human plasma samples were tested in the assay. Diluted samples were acetylated and run in the Acetylated Format. Values ranged from 9.0 to 16.27 pmol/mL with an average for the samples of 13.1 pmol/mL. The normal reference range for cAMP in plasma is 3.9-13.7 pmol/ mL10. Seven normal human urine samples were diluted > 1:30 in Sample Diluent and values ranged in the neat samples from 2,879 to 4,692 pmol/mL with an average for the samples of 3,690.1 pmol/ mL. The normal reference range for cAMP in urine is 800-12,000 pmol/mL11. Six normal human saliva samples were diluted 1:4 in Sample Diluent and run in both the Regular and Acetylated Formats. Values ranged from 4.91 to 15.07 pmol/mL with an average of 8.54 pmol/mL in the neat samples. The normal range for cAMP in saliva is 3.4-17.2 pmol/mL12.
Assay Time:	2.5 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. 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 stable 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 standard curve.

	A clear microtiter plate coated with an antibody to capture sheep IgG is provided and 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 substrate is added. The substrate reacts with the bound cAMP-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. 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 thaw and come to room temperature for 30-60 minutes. 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. ® www.ArborAssays.com 9 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 six test tubes as #1 through #6. Pipet 270 µL of Sample Diluent into tube #1 and 200 µL into tubes #2 to #6. the cyclic AMP stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 30 µL of the cAMP stock solution to tube #1 and vortex completely. Take 100 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 #6. The concentration of Cyclic AMP in tubes 1 through 6 will be 150, 50, 16.67, 5.56, 1.85, and 0.617 pmol/mL. non-Acetylated Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Sample Diluent (µl) 270 200 200 200 200 200 Addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Vol of Addition (µl) 30 100 100

	100 100 100 final conc (pM/ mL) 150 50 16.67 5.56 1.85 0.617 Use Standards within 1 hour of preparation. ® 10 EXPECT ASSAY ARTISTRY ASSAy Protocol - regUlAr forMAt We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine cAMP concentrations. 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 25 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 (Bo 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 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 TMB Substrate to each well, using a repeater. 11. Incubate the plate at room temperature for 30 minutes without shaking. 12. Add 50 µL of the Stop Solution to each well, using a repeater pipet. 13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. 14. 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 compatible 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:	Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD'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 MyAssays to calculate the data: www.myassays.com/arbor-assays-cyclic-amp-direct-eia-kit-non-acetyl.assay typiCal Data - RegulaR foRmat sample mean oD net oD % B/B0 Cyclic amp Conc. (pmol/mL) NSB 0.054 0 - - Standard 1 0.155 0.101 10.6 150 Standard 2 0.257 0.203 21.3 50 Standard 3 0.411 0.357 37.4 16.67 Standard 4 0.637 0.583 61.1 5.56 Standard 5 0.873 0.819 85.8 1.85 Standard 6 0.973 0.919 96.3 0.617 B0 1.008 0.954 100.0 0 Sample 1 0.510 0.456 47.8 10.4 Sample 2 0.634 0.580 60.7 6.0 ® *The MyAssays logo is a registered trademark of MyAssays Ltd. 12 EXPECT ASSAY ARTISTRY typical Standard curve - regular format 100 % 1.0 90 % 0.9 80 % 0.8 70 % 0.7 60 % 0.6 %B/B0 Net OD 50 % 0.5 %B/B0 40 % 0.4 30 % 0.3 20 % 0.2 10 % 0.1 0 % 0.0 0.1 1 10 100 1000 Cyclic AMP Conc. (pmol/mL) 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

Application Details

	comparing the OD's for nineteen wells run for each of the B0 and standard #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. Sensitivity was determined as 0.64 pmol/mL. The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human urine sample. limit of Detection was determined as 0.20 pmol/mL
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 laboratory 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 manufacturers 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 8. 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. The Stop Solution is 1M HCl. These solutions should not come in contact with skin or eyes. Take appropriate precautions when handling these reagents. The kit uses acetic anhydride and triethylamine as acetylation reagents. Triethylamine and acetic anhydride are lachrymators. 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:

Kurihara, Itoh, Shimizu, Walenna, Chou, Ishii, Soejima, Fujikane, Hiromatsu: "Chlamydia trachomatis targets mitochondrial dynamics to promote intracellular survival and proliferation. " in: **Cellular microbiology**, Vol. 21, Issue 1, pp. e12962, (2019) (PubMed).

Allouche-Fitoussi, Bakhshi, Breitbart: "Signaling pathways involved in human sperm hyperactivated motility stimulated by Zn2." in: **Molecular reproduction and development**, Vol. 85, Issue 6, pp. 543-556, (2018) (PubMed).

Xinhong, Zhen, Fu, Wang, Yang, Li, Li: "Quantitative proteomic profiling indicates the difference in reproductive efficiency between Meishan and Duroc boar spermatozoa." in: **Theriogenology**, Vol. 116, pp. 71-82, (2018) (PubMed).

Blain-Hartung, Rockwell, Moreno, Martin, Gan, Bryant, Lagarias: "Cyanobacteriochrome-based photoswitchable adenylyl cyclases (cPACs) for broad spectrum light regulation of cAMP levels in cells." in: **The Journal of biological chemistry**, Vol. 293, Issue 22, pp. 8473-8483, (2018) (PubMed).

Viola, Wearick-Silva, Creutzberg, Kestering-Ferreira, Orso, Centeno-Silva, Albrechet-Souza, Marshall, Li, Bredy, Riva, Grassi-Oliveira et al.: "Postnatal impoverished housing impairs adolescent risk-assessment and increases risk-taking: A sex-specific effect associated with histone epigenetic regulation of Crfr1 in the medial prefrontal ..." in: **Psychoneuroendocrinology**, Vol. 99, pp. 8-19, (2018) (PubMed).

There are more publications referencing this product on: Product page

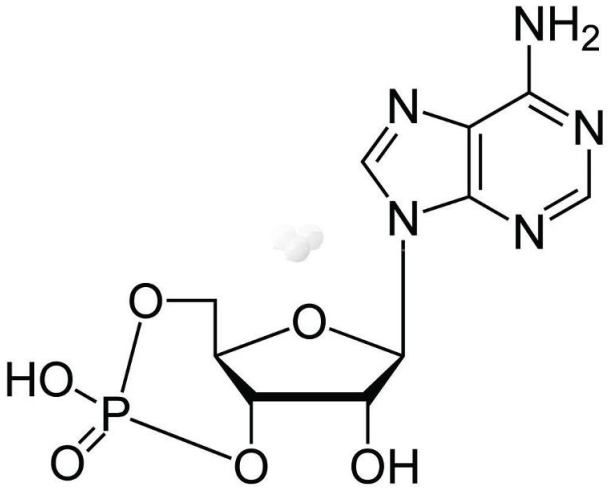

Image 1.

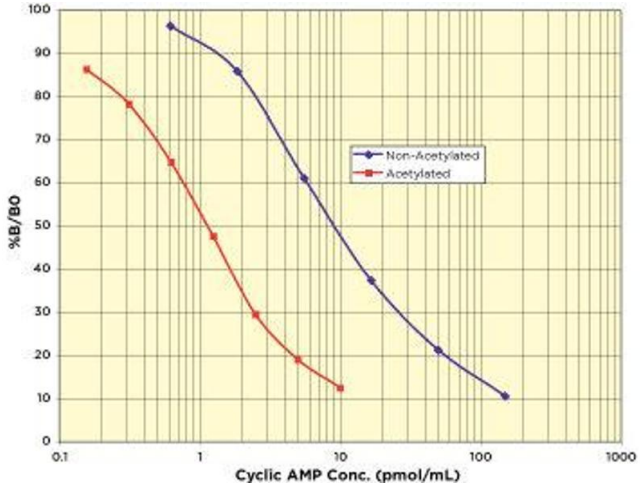

Image 2.

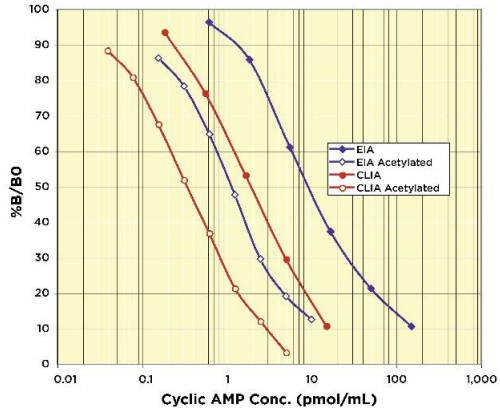

Image 3.

