KAMIYA BIOMEDICAL COMPANY

Cyclic AMP Direct EIA Kit

For the quantitative determination of cAMP in cell lysates, saliva, urine, EDTA and heparin plasma, tissue culture media

Cat. No. KT-717

For Research Use Only.

PRODUCT INFORMATION
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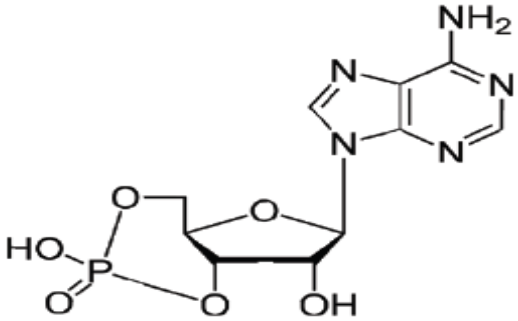
BACKGROUND
Adenosine-3',5'-cyclic monophosphate, or cyclic AMP (cAMP), $C_{10}H_{12}N_5O_6P$, is one of the most important second messengers and a key intracellular regulator. Discovered by Sutherland and Rall in 1957, it functions as a mediator of activity for a number of hormones, including epinephrine, 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.



Other biological actions of cAMP include regulation of innate immune functioning, axon regeneration, cancer, and inflammation.

PRINCIPLE
The Direct Cyclic AMP (cAMP) Immunoassay kit is designed to quantitatively measure cAMP present in lysed cells, EDTA and heparin plasma, urine, saliva and tissue culture media samples. Please read the complete kit insert before performing this assay.

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 calibrators 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 calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration 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. Calibrators or diluted samples, either with or without acetylation, are pipetted into the primed wells. A cAMP-peroxidase conjugate is added to the calibrators 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.

COMPONENTS
Coated Clear 96 Well Plates
A clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.
1 Each

Cyclic AMP Calibrator
Cyclic AMP at 1,500 pmol/mL in a special stabilizing solution.
125 µL

Cyclic AMP Antibody
A sheep antibody specific for cyclic AMP.
3 mL

Cyclic AMP Conjugate
A cyclic AMP-peroxidase conjugate in a special stabilizing solution.
3 mL

Sample Diluent
Contains special stabilizers and additives. One plate kit uses a ready-to-use Sample Diluent. Five plate kit uses a 4X concentrate that must be diluted with deionized or distilled water. **CAUSTIC**
28 mL

Plate Primer
A neutralizing solution containing special stabilizers and additivies.
5 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

TMB Substrate
11mL

Stop Solution
A 1M solution of hydrochloric acid. CAUSTIC.
5 mL

Plate Sealer
1 Each

STORAGE

All components of this kit should be stored at 4℃ until the expiration date of the kit.

OTHER MATERIALS REQUIRED

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. Contact your plate reader manufacturer for details.

PRECAUTIONS

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.

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.

SAMPLE TYPES

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 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 calibrators and samples should be diluted in glass test tubes.**

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 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 ~ 10^7 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.

Tissue Samples

Tissues samples should be frozen in liquid nitrogen and stored at -80°C if analysis is not to be carried out immediately.

Grind the frozen tissue in a stainless steel mortar under liquid nitrogen until it is a fine powder. Allow the liquid nitrogen to evaporate and weigh the powdered tissue. Add 1 mL of Sample Diluent for every 100 mg of tissue. Incubate in the Sample Diluent for 10 minutes on ice, and then centrifuge at ≥600 x g at 4°C for 15 minutes. Collect the supernatant and run in the assay immediately or store frozen at ≤ -70°C.

For samples that require concentration and delipidation, a trichloroacetic acid (TCA)/ether protocol can be used. Grind the frozen tissue in a stainless steel mortar under liquid nitrogen until it is a fine powder. Allow the liquid nitrogen to evaporate and weigh the powdered tissue. Add 1 mL of ice cold 5% TCA (weight/volume) for every 100 mg of tissue and grind in a glass-Teflon mortar. Incubate in the TCA for 10 minutes on ice, and then centrifuge at ≥600 x g at 4°C for 15 minutes. Collect the supernatant.

For every 1 mL of TCA supernatant add 3 mL of water saturated diethyl ether* and shake in a glass vial. Allow the ether to separate as the top layer, remove it and discard the ether. Dry the aqueous layer by lyophilization or using a vacuum centrifuge. Reconstitute by adding 1 mL of Sample Diluent for every mL of 5% TCA used to extract and run in the assay immediately or store at ≤ -70°C.
***Diethyl ether is extremely flammable and should be used in a hood.**

Tissue Culture Media
For measuring cAMP in tissue culture media (TCM), samples should be read off a calibration curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Plasma Samples
Plasma samples should be diluted ≥ 1:10 with the supplied Sample Diluent and acetylated prior to running in the Acetylated Format assay.

Urine Samples
Urine samples should be diluted ≥ 1:20 with the supplied Sample Diluent prior running in the assay. Due to the high concentration of cAMP in urine, samples may need to be diluted further.

Saliva Samples
Saliva samples should be diluted ≥ 1:4 with the supplied Sample Diluent prior running in the assay.

Use all samples within 2 hours of dilution in Sample Diluent.

REAGENT PREPARATION

Allow the kit reagents to thaw and come to room temperature for 30-60 minutes. We recommend that all calibrators 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.

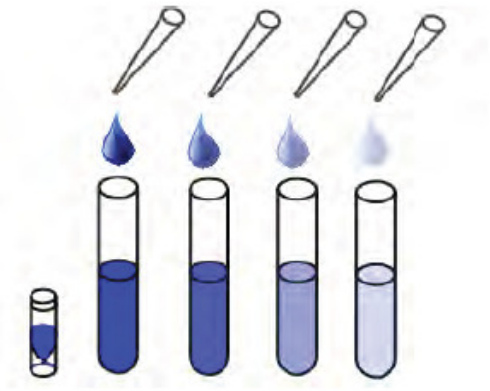
Wash Buffer
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

REAGENT PREPARATION - REGULAR FORMAT

Use this format for urine, saliva and some cell lysates. Do NOT use for plasma samples.

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

Calibrator 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 Calibrators within 1 hour of preparation.

ASSAY PROTOCOL - REGULAR FORMAT

1. Use the plate layout sheet on the back page to aid in proper sample and calibrator 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. F**ailure** To A**dd** P**late** P**rimer** To ALL **Well**s F**irst Will** C**ause** Ass**ay** To F**ail.**

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 calibrators into wells in the plate.

NOTE: Sample Diluent will turn from orange to bright pink upon sample or calibrator addition to the Plate Primer in the wells.

6. Add 25 µL of the cAMP Conjugate to each well using a repeater pipet.

7. Add 25 µL of the 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.

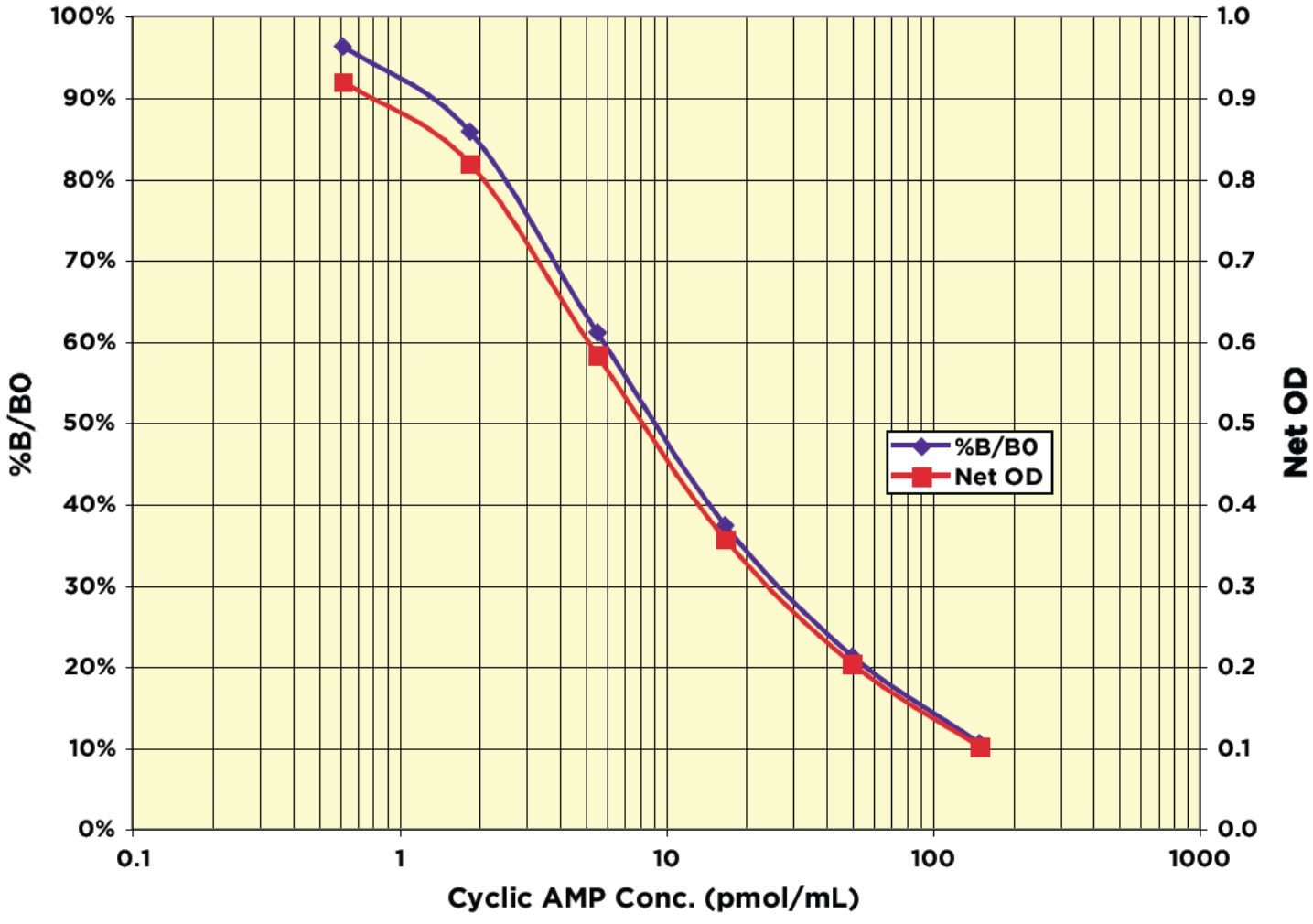
CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration 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.

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

TYPICAL CALIBRATION CURVE - REGULAR FORMAT



Always run your own calibration 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 OD's for nineteen wells run for each of the B0 and calibrator #6. The detection limit was determined at two (2) standard deviations from the B0 along the calibration 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 calibrator and a low concentration human urine sample.
Limit of Detection was determined as 0.20 pmol/mL.

ACETYLATED PROTOCOL - OVERVIEW

Use this format for plasma, some cell lysates and any sample with low cAMP concentrations.

Prior to running the acetylated assay, all calibrators, samples and the Sample Diluent used for the B0 and NSB wells must be acetylated. Acetylation is carried out by adding 10 µL of the Acetylation Reagent (as prepared below) for each 200 µL of the calibrator, sample and Sample Diluent. Vortex each treated calibrator, sample or Sample Diluent after addition of the Acetylation Reagent and **use within 30 minutes of preparation**.

Note: Upon Acetylation, all of the calibrators 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.

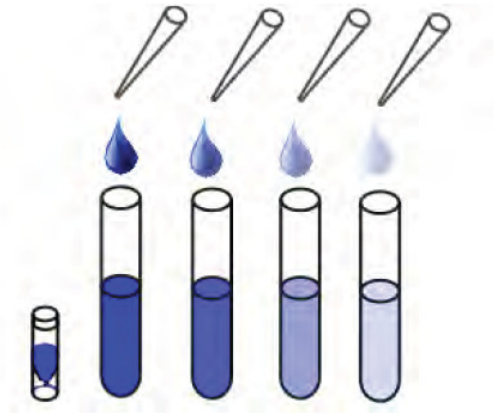
Reagents	Number of Samples to be Tested			
	20	40	100	200
Acetic Anhydride Volume (µL)	200	400	1,000	2,000
Triethylamine Volume (µL)	400	800	2,000	4,000
Acetylation Reagent Vol (mL)	0.6	1.2	3	6

Use the Acetylation Reagent within 60 minutes of preparation.

Calibrator Preparation - Acetylated

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

Label seven test tubes as #1 through #7. Label one tube as Stock Dilution. Pipet 270 µL of Sample Diluent into the Stock Dilution tube. Pipet 560 µL of Sample Diluent into tube #1 and 300 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 the Stock Dilution tube and vortex completely. Carefully add 40 µL of the Stock Dilution tube to tube #1 and vortex completely. Take 300 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 #7. The concentration of Cyclic AMP in tubes 1 through 7 will be 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 pmol/mL.



	Stock Dil'n	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Sample Diluent (µL)	270	560	300	300	300	300	300	300
Addition	Stock	Stock Dil'n	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	30	40	300	300	300	300	300	300
Final Conc (pmol/mL)	150	10	5	2.5	1.25	0.625	0.313	0.156

Calibrator and Sample Acetylation

Pipet 300 µL of Sample Diluent into a glass tube to act as the Zero calibrator/NSB tube. Add 15 µL of Acetylation Reagent to this tube and vortex immediately. Proceed to assay within 30 minutes.

Pipet 200 µL of each calibrator or sample to be tested into glass tubes. Add 10 µL of the Acetylation Reagent into each tube and vortex immediately. Proceed to assay within 30 minutes.

NOTE: Samples and Sample Diluent will turn from orange to pale yellow upon acetylation.

Use Acetylated Calibrators and Samples within 30 minutes of preparation.

ASSAY PROTOCOL - ACETYLATED FORMAT

1. Use the plate layout sheet on the back page to aid in proper sample and calibrator 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. F**ailure** To A**dd** P**late** P**rimer** To ALL **Well**s F**irst Will** C**ause** Ass**ay** To F**ail.**

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

4. Pipet 50 µL of acetylated Sample Diluent into wells to act as maximum binding wells (Bo or 0 pg/mL).

5. Pipet 50 µL of acetylated samples or calibrators into wells in the plate.

6. Add 25 µL of the cAMP Conjugate to each well using a repeater pipet.

7. Add 25 µL of the 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.

Note: Wells will have turned from very pale yellow to pale pink during incubation.

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 pipet.

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.

CALCULATION OF RESULTS

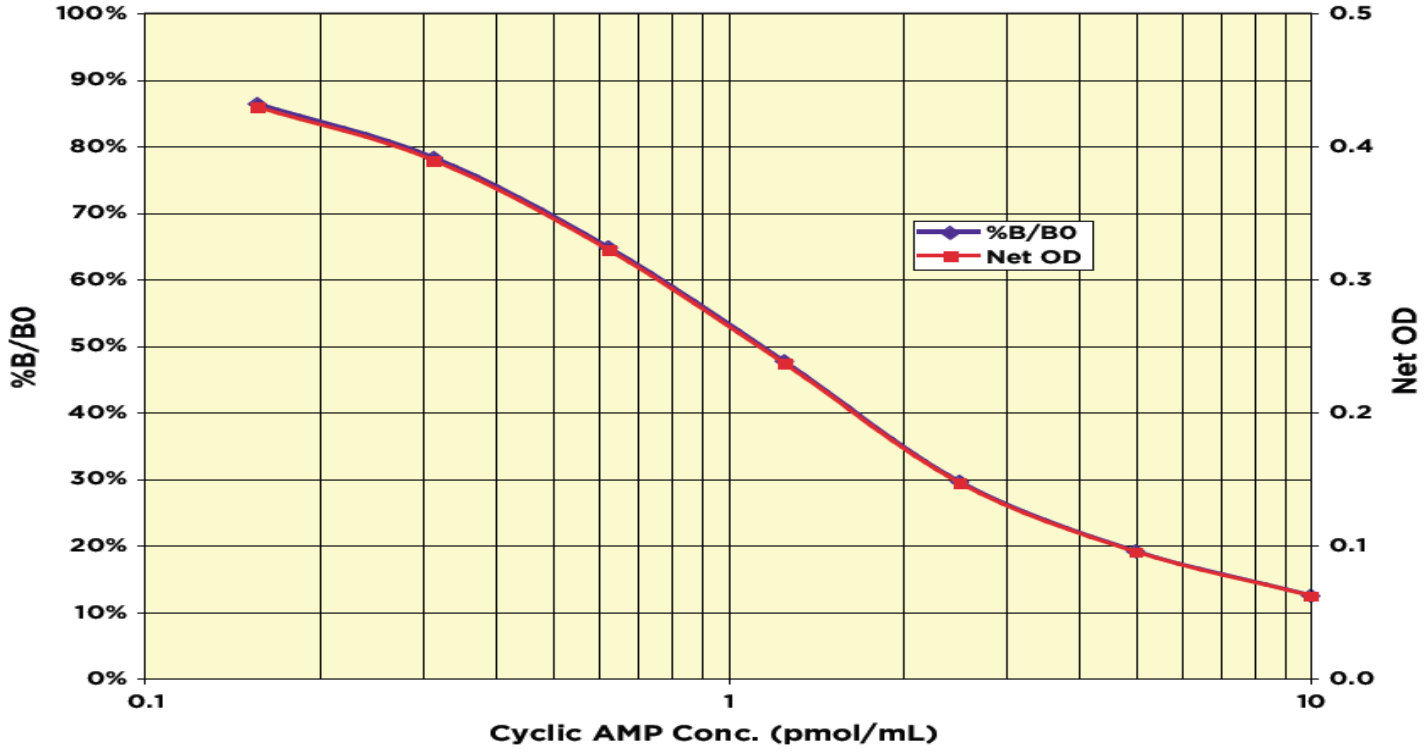
Average the duplicate OD readings for each calibrator and sample. Create a calibration curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

TYPICAL DATA - ACETYLATED

Sample	Mean OD	Net OD	% B/B0	Cyclic AMP Conc. (pmol/mL)
NSB	0.045	0	-	-
Standard 1	0.107	0.062	12.5	10
Standard 2	0.140	0.095	19.1	5
Standard 3	0.192	0.147	29.6	2.5
Standard 4	0.282	0.237	47.7	1.25
Standard 5	0.367	0.322	64.8	0.625
Standard 6	0.434	0.389	78.3	0.3125
Standard 7	0.474	0.429	86.3	0.156
B0	0.542	0.497	100.0	0
Sample 1	0.231	0.186	37.4	1.820
Sample 2	0.369	0.324	65.2	0.622

Always run your own calibration curve for calculation of results. Do not use this data.

TYPICAL CALIBRATION CURVE - ACETYLATED



Always run your own calibration curve for calculation of results. Do not use this data.

VALIDATION DATA - ACETYLATED FORMAT

Sensitivity and Limit of Detection - Acetylated
Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the acetylated B0 and calibrator #6. The detection limit was determined at two (2) standard deviations from the B0 along the calibration curve.

Sensitivity was determined as 0.083 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 acetylated zero calibrator and a low concentration acetylated human sample.
Limit of Detection was determined as 0.078 pmol/mL. This is equivalent to 3.9 fmol cAMP per sample.

VALIDATION DATA - REGULAR AND ACETYLATED

Linearity
Linearity was determined by taking two human urine samples, one with a low cAMP level of 10.7 pmol/mL and one with a higher level of 91.5 pmol/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Serum	Low Serum	Observed Conc. (pmol/mL)	Expected Conc. (pmol/mL)	% Recovery
80%	20%	63.6	75.4	84.4
60%	40%	47.4	59.2	80.1
40%	60%	41.7	43.0	97.0
20%	80%	30.9	26.9	114.9
			Mean Recovery	**94.1%**

Intra Assay Precision - Regular
Three human urine samples were diluted with Sample Diluent and run in replicates of 20 in an assay. The mean and precision of the calculated cAMP concentrations were:

Sample	Cyclic AMP Conc. (pmol/mL)	%CV
1	56.9	8.6
2	11.9	11.3
3	6.7	12.3

Inter Assay Precision - Regular
Three human urine samples were diluted with Sample Diluent and run in duplicates in twelve assays run over multiple days by four operators. The mean and precision of the calculated cAMP concentrations were:

Sample	Cyclic AMP Conc. (pmol/mL)	%CV
1	57.1	10.0
2	10.9	11.5
3	6.3	11.3

Intra Assay Precision - Acetylated
Two human plasma samples were diluted with Sample Diluent, acetylated and run in replicates of 20 in an assay. The mean and precision of the calculated cAMP concentrations were:

Sample	Cyclic AMP Conc. (pmol/mL)	%CV
1	1.74	10.4
2	0.60	11.8

Inter Assay Precision - Acetylated
One human urine and two human plasma sample were diluted with Sample Diluent, acetylated and run in duplicates in twelve assays run over multiple days by four operators. The mean and precision of the calculated cAMP concentrations were:

Sample	Cyclic AMP Conc. (pmol/mL)	%CV
1	5.22	9.8
2	1.99	8.1
3	0.73	15.4

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/mL. 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/mL. 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/mL.

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Nucleotide	Cross Reactivity (%)
Cyclic AMP	100%
AMP	< 0.08%
GMP	< 0.08%
Cyclic GMP	< 0.08%
ATP	< 0.08%

INTERFERENTS

A variety of detergents were tested as possible interfering substances in the assay. CHAPS, and Tween 20 at 0.1% increased measured cAMP by 8.9 and decreased measured cAMP by 0.9% respectively. Triton X-100 at 2% increased measured cAMP by 1.8% and CTAC at 0.05% increased measured cAMP by 6.3%. Samples containing SDS above 0.01% should not be used in the assay.

FOR RESEARCH USE ONLY

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