FT-1B7080

Cyclic AMP High Sensitivity Chemiluminescent Assay Kit

Species independent cAMP Direct Assay Kit

Product Description

Name :	**cAMP High Sensitivity Chemiluminescent Assay Kit**
Catalog Number :	FP-1B7080, 1 x 96-plate
	FP-1B7081, 5 x 96-plate
Sample types :	Cell Lysates, Saliva, Urine, EDTA and Heparin Plasma, Tissue Culture Media

Storage: All components of this kit should be stored at 4°C until the expiration date of the kit.

Introduction

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[1], it functions as a mediator of activity for a number of hormones, including epinephrine, glucagon, and ACTH[2-4]. 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 cAMP[5].

Other biological actions of cAMP include regulation of innate immune functioning[6], axon regeneration[7], cancer[8], and inflammation[9].

1. Sutherland, E. W. and Rall, T. W. Fractionation and Characterization of a Cyclic Adenine Ribonucleotide Formed by Tissue Particles. J. Biol. Chem., 232:1077, 1958.
2. Marsh, J.M., The Role of Cyclic AMP in Gonadal Steroidogenesis. Biol. Reprod., 14:30-53, 1976.
3. Korenman, S.G. and Krall, J.F., The Role of Cyclic AMP in the Regulation of Smooth Muscle Cell Contraction in the Uterus. Biol. Reprod., 16:1-17, 1977.
4. Kelley, D.J., Bhattacharyya, A., Lahvis, G.P., Yin, J.C.P., Malter, J., and Davidson, R.J., The Cyclic AMP Phenotype of Fragile X and Autism. Neurosci. Biobehav. Rev., 32(8):1533-1543, 2008.
5. http://www.hmdb.ca/metabolites/HMDB00058
6. Serezani, C.H., Ballinger, M.N., Aronoff, D.M., and Peters-Golden, M., Cyclic AMP. Master Regulator of Innate Immune Cell Function. Am. J. Resp. Cell and Mol. Biol., 39 (2): 127, 2008.
7. Hannila, S.S., and Filbin, M.T., The role of cyclic AMP signaling in promoting axonal regeneration after spinal cord injury. Exp. Neurol., 209(2): 321–332, 2008.
8. Shankar, D.B, Cheng, J.C., and Sakamoto, K.M., Role of cyclic AMP response element binding protein in human leukemias. Cancer, 104(9):1819-24, 2005.
9. Galea E. and Feinstein, D.L., Regulation of the expression of the inflammatory nitric oxide synthase (NOS2) by cyclic AMP. FASEB J., 13:2125-2137, 1999.

Directions for use

Assay Principle

The Cyclic AMP (cAMP) High Sensitivity Chemiluminescent Assay 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. 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 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 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.

Supplied Components

Coated White 96 Well Plates 1 or 5 plate(s)
White plastic microtiter plate(s) coated with donkey anti-sheep IgG.

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

Cyclic AMP Antibody 3 mL or 13 mL
A sheep antibody specific for cyclic AMP.

Cyclic AMP Conjugate Concentrate 60 µL or 260 µL
A 50X cyclic AMP-peroxidase conjugate concentrate stock in a special stabilizing solution.

Conjugate Diluent 3 mL or 13 mL
Contains special stabilizers and additives.

Sample Diluent 12 mL or 60 mL **Now Supplied ONLY as Concentrate**
Contains special stabilizers and additives.
This 4X concentrate must be diluted with deionized or distilled water. **CAUSTIC**

Plate Primer 5 mL or 25 mL
A neutralizing solution containing special stabilizers and additivies.

Acetic Anhydride 2mL
WARNING: Corrosive Lachrymator

Triethylamine 4mL
WARNING: Corrosive Lachrymator

Wash Buffer Concentrate 30 mL or 125 mL
A 20X concentrate that must be diluted with deionized or distilled water.

Substrate Solution A 6mL or 28 mL

Substrate Solution B 6mL or 28 mL

Plate Sealer 1 or 5 Each

Other Materials Required

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.
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 100µL of prepared CLIA substrate (see page 8 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. 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 as prepared previously.
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 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 (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.**

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 standard 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.

Salive 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.

FT-1B7080

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 Suplied ONLY as Concentrate

Prepare the Sample Diluent by diluting the Sample Diluent Concentrate (60mL) 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

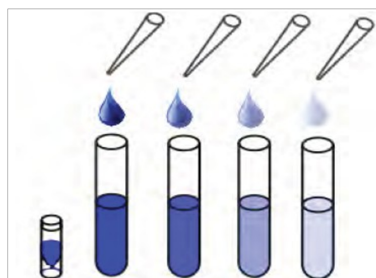
Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle.
Once mixed the substrate is stable for one month when stored at 4°C.

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 Diluent 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.



FT-1B7080

Non-Acetylated	Stock 2	Std 1	Std 2	Std 3	Std 4	Std 5
Sample Diluent (µL)	**90**	**450**	200	200	200	200
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

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 (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 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 mixed Chemiluminescent Substrate to each well, using a repeater.
11. Immediately 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.
12. Use the plate reader's built-in 4PLC software capabilities to calculate cAMP concentration for each sample.

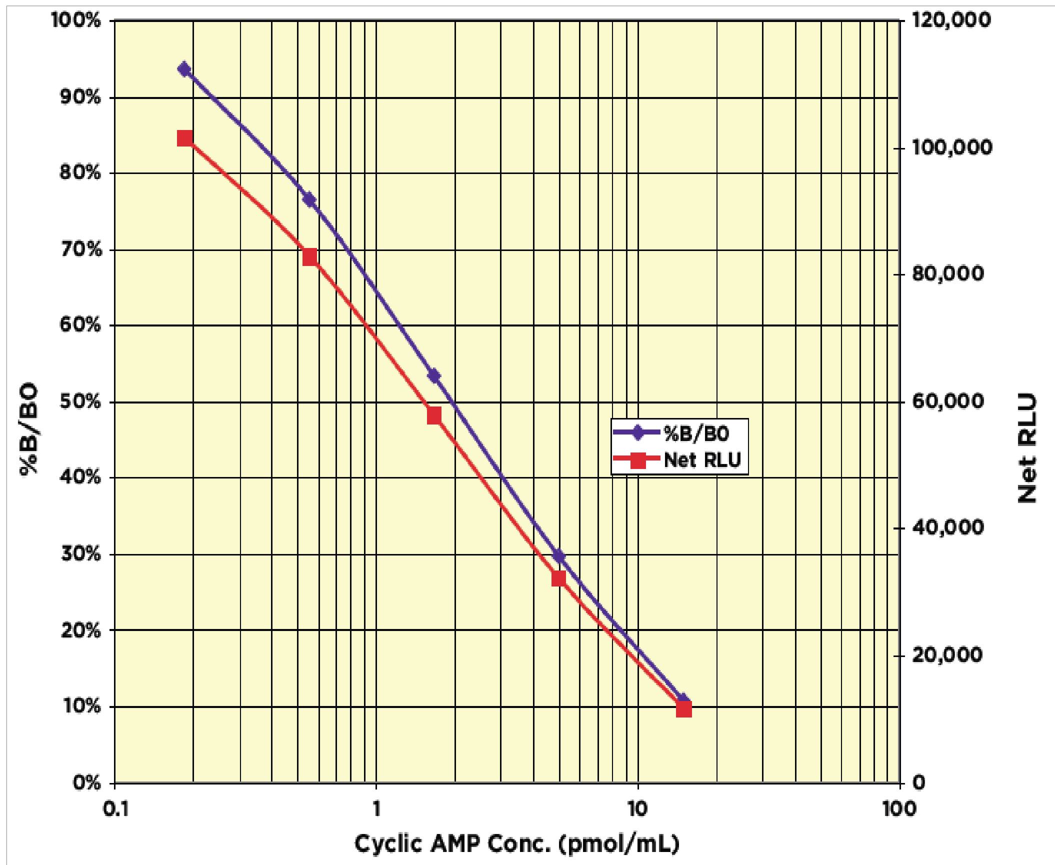
Calculation of results

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. Create 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.

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	53.5	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

Typical standard curve - regular format



**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.
Limit of Detection was determined as 0.076 pmol/mL. This is equivalent to 3.8 fmol cAMP per well.

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 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 Acetylation 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

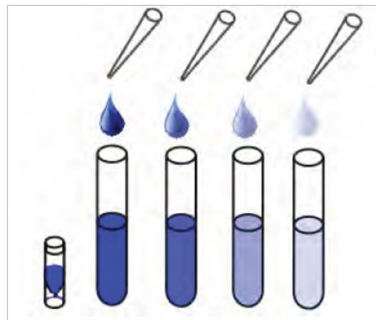
Working in a fume hood mix one part of Acetic Anhydride with 2 parts of Triethylamine in a glass test tube. Use the following table to help determine the amount of Acetylation Reagent to make.

Reagents	Number of Samples to be Tested			
	20	**40**	**100**	**200**
Acetic Anhydride Volume (µL)	200	400	1000	2000
Triethylamine Volume (µL)	400	800	2000	4000
Acetylation Reagent Vol (mL)	**0,6**	**1,2**	**3**	**6**

Use the Acetylation Reagent within 60 minutes of preparation.

Standard Preparation - Acetylated

All standards and samples should be diluted in glass test tubes.
Label seven test tubes as #1 through #8. Label one tube as Stock 2. Pipet 290 µL of Sample Diluent into the Stock 2 tube. Pipet 900 µL of Sample Diluent into tube #1 and 400 µL into tubes #2 to #8. **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. Carefully add 100µL of the Stock 2 tube to tube #1 and vortex completely. Take 400 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 #8. The concentration of Cyclic AMP in tubes 1 through 8 will be 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, and 0.039 pmol/mL.



	Stock 2	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Sample Diluent (µL)	**290**	**900**	400	400	400	400	400	400	400
Addition	cAMP Std.	Stock 2	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**10**	**100**	400	400	400	400	400	400	400
Final Conc (pmol/mL)	50	5	2.5	1.25	0.625	0.313	0.156	0.078	0.039

Standard and Sample Acetylation

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

Pipet 300 µL of each standard or sample to be tested into glass tubes. Add 15 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 Standards and Samples within 30 minutes of preparation.

FT-1B7080

Assay protocol - acetylated 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 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 standards 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 mixed Chemiluminescent Substrate to each well, using a repeater pipet.
11. Immediately 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.

12. Use the plate reader's built-in 4PLC software capabilities to calculate cAMP concentration for each sample.

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. Create 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.

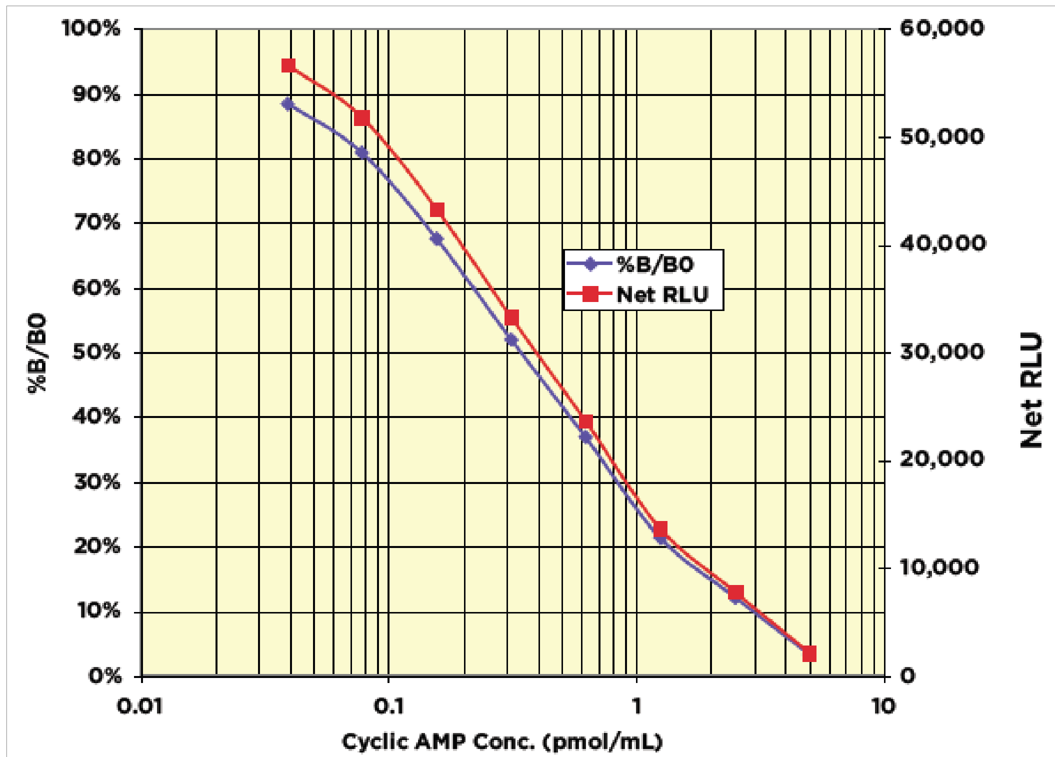
Typical data - acetylated

Sample	Mean RLU	Net RLU	% B/B0	Cyclic AMP Conc. (pmol/mL)
NSB	11 735	O	-	-
Standard 1	13 840	2 105	3.3	5
Standard 2	19 495	7 760	12.1	2.5
Standard 3	25 385	13 650	21.3	1.25
Standard 4	35 390	23 655	36.9	0.625
Standard 5	45 000	33 265	51.9	0.3125
Standard 6	55 030	43 295	67.6	0.156
Standard 7	63 545	51 810	80.9	0.078
Standard 8	68 355	56 620	88.4	0.039
B0	75 775	64 040	100,0	0
Sample 1	28 830	17 095	26.7	0.98
Sample 2	45 310	33 575	52.4	0.310

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

FT-1B7080

Typical Standard Curve – Acetylated



Always run your own standard 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 RLU's for eighteen wells run for each of the acetylated B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 0.015 pmol/mL. This is equivalent to 0.75 fmol cAMP per sample.

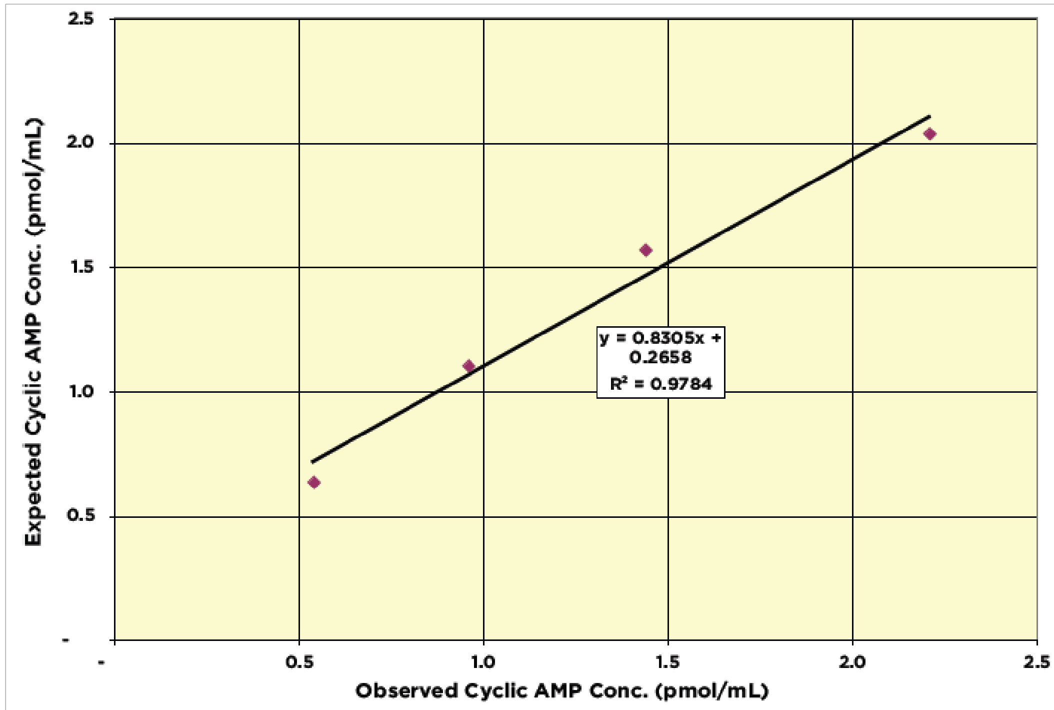
The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty runs for each of acetylated zero standard and a low concentration acetylated human sample.
Limit of Detection was determined as 0.019 pmol/mL. This is equivalent to 0.95 fmol cAMP per sample.

Validation data - regular and acetylated

Linearity

Linearity was determined by taking two Jurkat cell lysate samples, one with a low cAMP level of 0.2 pmol/mL and one with a higher level of 2.5 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	High Serum	Observed Conc. (pmol/mL)	Expected Conc. (pmol/mL)	% Recovery
80%	20%	2.2	2.0	108.7
60%	40%	1.4	1.6	91.8
40%	60%	1.0	1.1	87.1
20%	80%	0.5	0.6	84.9
			Mean Recovery	**93.1%**



Intra Assay Precision - Regular

Two 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	11.2	3.9
2	3.6	8.3

Inter Assay Precision - Regular

Two human urine samples were diluted with Sample Diluent and run in duplicates in fourteen 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	6.5	8.1
2	2.1	13.0

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	1.32	8.4
2	0.39	10.9

Inter Assay Precision - Acetylated

Two human plasma sample were diluted with Sample Diluent, acetylated and run in duplicates in fourteen assays run over multiple days by four operators. The mean and precision of the calculated cAMP concentrations were:

FT-1B7080

Sample	Cyclic AMP Conc. (pmol/mL)	%CV
1	1.02	10.1
2	0.32	16.5

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 average for the samples of 21.15 pmol/mL. The normal reference range for cAMP in plasma is 3.9-13.7 pmol/mL[10]. 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/mL[11]. 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/mL[12].

10. NIH Clinical Center, http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index/EB6E90F8D951346F85256BA4004C96E4?OpenDocument
11. NIH Clinical Center, http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index/24B381AEE513EB8785256BA40052ADAD?OpenDocument
12. Sproles, A.C., Cyclic AMP Concentration in Saliva of Normal Children and Children with Down's Syndrome, J. Dent. Res., 1976, 52, 915-917.

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.

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H	G	F	E	D	C	B	A	
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