bio-techne®

R&D SYSTEMS

Parameter™

cAMP Assay

Catalog Number KGE002B
SKGE002B
PKGE002B

For the quantitative determination of cyclic AMP (cAMP) concentrations in cell culture supernates, cell lysates, serum, plasma, saliva, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Adenosine 3'5'-cyclic monophosphate (cAMP) is a ubiquitous second messenger that mediates a diverse range of cellular processes in all organisms from bacteria to higher eukaryotes (1, 2). It is converted from adenosine triphosphate (ATP) by adenylyl cyclases (ACs) (3, 4), and is inactivated by phosphodiesterases (PDEs) which catalyze its hydrolysis to 5'-AMP (5). In mammals, upon interaction with extracellular ligands, G protein coupled receptors (GPCRs) linked to $G\alpha_s$ activate the family of nine transmembrane ACs to increase intracellular cAMP. In contrast, GPCRs associated with $G\alpha_{i/o}$ inhibit the synthesis of cAMP by transmembrane ACs. With the exception of AC9, transmembrane ACs can be activated by forskolin, a plant diterpene commonly used to raise cAMP levels in cells (6). A divalent cation-dependent soluble AC (sAC) isoform also exists. It is activated by bicarbonate ions and can be found in the cytosol and in subcellular organelles (7).

The physiological roles of cAMP are mediated via multiple effector molecules (8). Binding of cAMP to protein kinase A (PKA) holoenzyme induces conformational changes and releases the catalytic subunit to phosphorylate target substrates on serine/threonine residues. cAMP binding to the guanine nucleotide exchange factors Epac1/2 (exchange protein activated by cAMP, also known as cAMP-GEF-I and cAMP-GEF-II) mediates the exchange of GDP for GTP on the small molecular weight G proteins Rap-1 and -2. Activated Rap proteins are important in multiple cellular processes including adhesion and exocytosis (8-10). cAMP can also activate cyclic nucleotide-gated ion channels (CNG) by binding directly to the nonselective cation channel proteins that are expressed in various tissues (8, 11). cAMP signaling is spatially and temporally regulated, allowing for the selective activation of a subset of targets. A-kinase anchoring proteins (AKAPs) provide the platform for the assembly of signalsomes consisting of cAMP effectors (PKA and/or Epac) and their substrates, together with signal terminators including phosphatases and PDEs (12-15).

In response to increases in intracellular cAMP, a wide variety of cell types possess mechanisms for exporting cAMP to the extracellular space (16-19). In mammals, plasma and urine cAMP concentrations can become highly elevated under certain physiological conditions. Extracellular cAMP is known to exert physiological actions on diverse cell types, in part through the cAMP-adenosine pathway where cAMP is converted to adenosine via ecto-PDEs and ectonucleotidases.

The R&D Systems® cAMP Immunoassay is a 3.5 hour competitive enzyme immunoassay designed to measure cAMP in cell culture supernates, cell lysates, serum, plasma, saliva, and urine.

PRINCIPLE OF THE ASSAY

This assay is based on the competitive binding technique. A monoclonal antibody specific for cAMP binds to the goat anti-mouse antibody coated onto the microplate. Following a wash to remove excess monoclonal antibody, cAMP present in a sample competes with a fixed amount of horseradish peroxidase (HRP)-labeled cAMP for sites on the monoclonal antibody. This is followed by another wash to remove excess conjugate and unbound sample. A substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of cAMP in the sample.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Standards, controls, and samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent or Cell Lysis Buffer and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
- **Samples containing mouse or rat IgG may interfere with this assay. Refer to the Mouse/Rat cAMP Parameter Assay (R&D Systems®, Catalog # KGE012) kit to measure mouse and rat samples.**

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Pre-rinse the pipette tip when pipetting.
- Pipette standards and samples to the bottom of the wells. Add all other reagents to the side of the wells to avoid contamination.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at ≤ -20 °C in a manual defrost freezer. Do not use past kit expiration date.

PART	PART #	CATALOG # KGE002B	CATALOG # SKGE002B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Goat Anti-mouse Microplate	892575	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a goat anti-mouse polyclonal antibody.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Cell Lysis Buffer 5	895890	1 vial	6 vials	15 mL/vial of a concentrated buffered solution with preservatives. *Use diluted 1:5 in this assay. May contain crystals. Warm to room temperature and mix well to dissolve. May turn yellow over time.*	Prepare fresh for each use. Discard the (1X) buffer after use.
cAMP Conjugate	893351	1 vial	6 vials	6 mL/vial of cAMP conjugated to horseradish peroxidase with red dye and preservatives.	May be stored for up to 1 month at 2-8 °C.*
cAMP Standard	893353	1 vial	6 vials	cAMP in buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Primary Antibody Solution	893352	1 vial	6 vials	6 mL/vial of a mouse monoclonal antibody to cAMP in buffer with blue dye and preservatives.	
Calibrator Diluent RD5-55	895398	2 vials	12 vials	15 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895209	2 vials	12 vials	11 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12.5 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	2 vials	12 vials	6 mL/vial of 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

KGE002B contains sufficient materials to run an ELISA on one 96 well plate.
SKGE002B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PKGE002B). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Goat Anti-mouse Microplate	892575	50 plates
Cell Lysis Buffer 5	895890	50 vials
cAMP Conjugate	893351	50 vials
cAMP Standard	893353	50 vials
Primary Antibody Solution	893352	50 vials
Calibrator Diluent RD5-55	895398	100 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	100 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

PRECAUTIONS

cAMP is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 50 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Collection device for saliva samples (Salivette® or equivalent)
- Test tubes for dilution of standards and samples
- cAMP Controls (optional; R&D Systems®, Catalog # QC52)

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing mouse or rat IgG may interfere with this assay.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Phosphodiesterase (PDE) inhibitors used in cell culture media may interfere in the assay. If a PDE inhibitor is used, precautions should be taken to prepare the appropriate control and run the control in the immunoassay to determine the effect of the inhibitor on the assay results.*

Cell Lysates - Prior to assay, cells must be lysed according to the directions in the Cell Lysis Procedure section.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell Culture Supernate/Saliva - Cell culture supernate and saliva samples require a 2-fold dilution. A suggested 2-fold dilution is 200 µL of sample + 200 µL of Calibrator Diluent RD5-55.

Serum/Plasma - Serum and plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 100 µL of sample + 400 µL of Calibrator Diluent RD5-55.

Urine - Urine samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-55.

CELL LYSIS PROCEDURE

1. Wash the cells three times in cold PBS.
2. Resuspend the cells in Cell Lysis Buffer 5 (diluted 1:5)* to a concentration of 1 x 10^7 cells/mL.
3. Freeze the cells at ≤ -20 °C. Thaw the cells with gentle mixing. Repeat the freeze/thaw cycle once. Trypan Blue and a microscope can be used to confirm cell lysis. Lysed cells will be blue. If cells are not lysed, repeat the freeze/thaw cycle as needed.
4. Centrifuge at 600 x g for 10 minutes at 2-8 °C to remove cellular debris.
5. Assay the supernate immediately, or aliquot and store at ≤ -20 °C.

The above method can be modified for Tissue Culture plates (6, 12, 24, 48, or 96 well). Incubate cells in the appropriate media overnight in a 37 °C, 5% CO_2 incubator prior to step 1.

A minimum of 250 µL of cell lysate is required to perform the assay in duplicate. The standard curve must be made in Cell Lysis Buffer 5 (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *cAMP is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

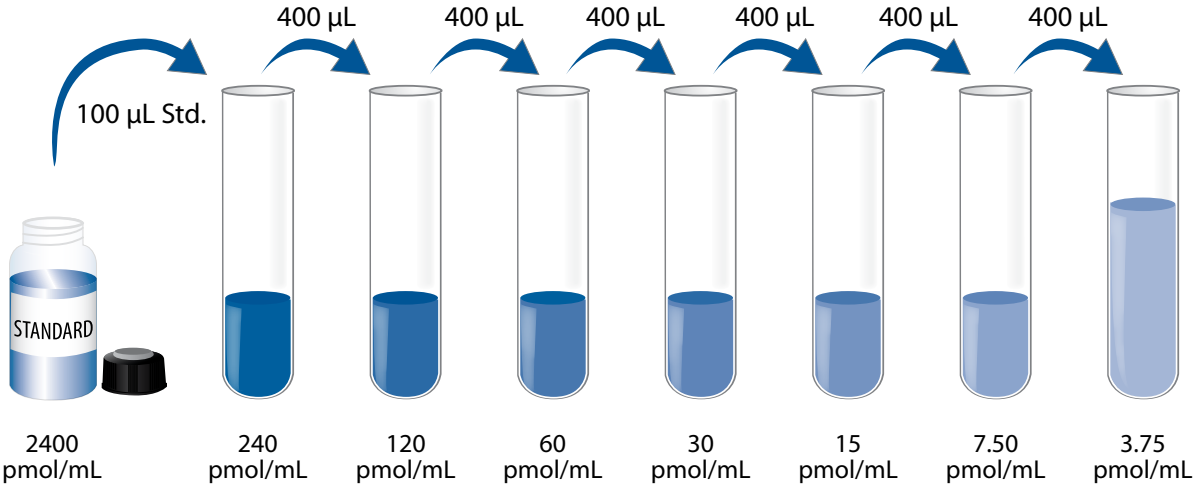
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Cell Lysis Buffer 5 (diluted 1:5) - Add 10 mL of Cell Lysis Buffer 5 to 40 mL of deionized or distilled water to prepare 50 mL of Cell Lysis Buffer 5 (diluted 1:5). *May contain crystals. Warm to room temperature and mix well to dissolve before diluting.*

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

cAMP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the cAMP Standard with deionized or distilled water. This reconstitution produces a stock solution of 2400 pmol/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-55 (*for cell culture supernate, serum, plasma, saliva, and urine samples*) or Cell Lysis Buffer 5 (diluted 1:5) (*for cell lysate samples*) into the 240 pmol/mL tube. Pipette 400 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 240 pmol/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (B_0) (0 pmol/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: *cAMP is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Primary Antibody Solution to all wells **except** the non-specific binding (NSB) wells. Cover with the adhesive strip provided, and incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm ± 50 rpm.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

   Note: *Do not allow wells to dry before addition of the cAMP Conjugate.*
5. Add 50 µL of cAMP Conjugate to all wells.
6. Add 100 µL of standard, control, or sample* to the appropriate wells within 15 minutes of addition of the cAMP Conjugate.
7. Add 100 µL of the appropriate diluent (Calibrator Diluent RD5-55 or Cell Lysis Buffer 5 (1X)) to the NSB and zero standard (B_0) wells. Cover with a new adhesive strip, and incubate for 2 hours at room temperature on the shaker.
8. Repeat the aspiration/wash as in step 4.
9. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution or lysis. See the Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample then subtract the average NSB optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis and draw the best fit curve through the points on the graph. Do not include the B_0 in the standard curve.

If desired, % B/B_0 can be calculated by dividing the corrected O.D. for each standard or sample by the corrected B_0 O.D. and multiplying by 100.

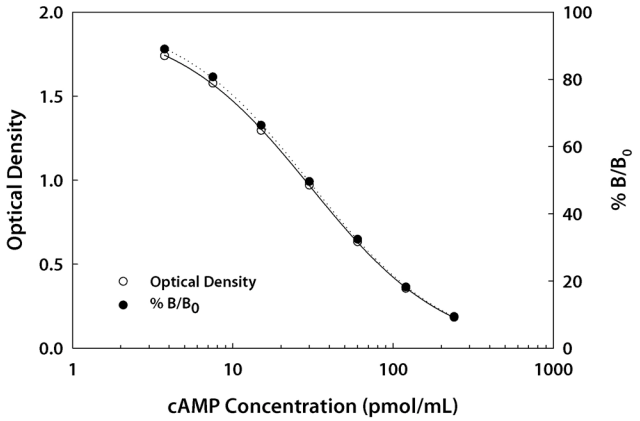
Calculate the concentration of cAMP corresponding to the mean absorbance from the standard curve.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

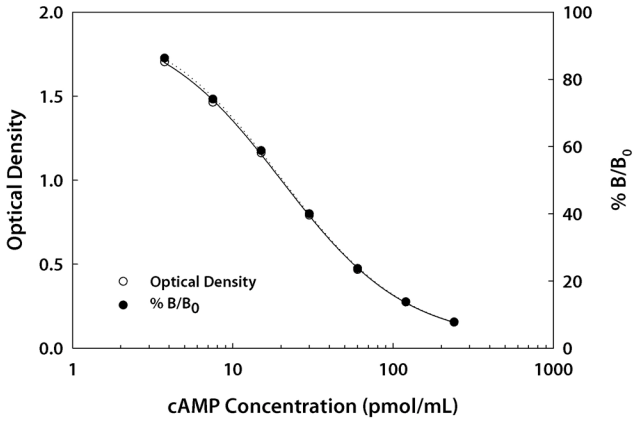
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATOR DILUENT RD5-55



(pmol/mL)	O.D.	Average	Corrected	% B/B_0
NSB	0.007 0.007	0.007	—	—
0 (B_0)	1.954 1.968	1.961	1.954	—
3.75	1.743 1.750	1.747	1.740	89.0
7.50	1.575 1.592	1.584	1.577	80.7
15	1.301 1.304	1.303	1.296	66.3
30	0.966 0.988	0.977	0.970	49.6
60	0.624 0.655	0.640	0.633	32.4
120	0.362 0.364	0.363	0.356	18.2
240	0.185 0.195	0.190	0.183	9.4

CELL LYSIS BUFFER 5 (diluted 1:5)



(pmol/mL)	O.D.	Average	Corrected	% B/B_0
NSB	0.006 0.006	0.006	—	—
0 (B_0)	1.965 1.997	1.981	1.975	—
3.75	1.667 1.752	1.710	1.704	86.3
7.50	1.456 1.481	1.469	1.463	74.1
15	1.151 1.184	1.168	1.162	58.8
30	0.783 0.810	0.797	0.791	40.0
60	0.472 0.476	0.474	0.468	23.7
120	0.276 0.283	0.280	0.274	13.8
240	0.153 0.166	0.160	0.154	7.8

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SERUM/PLASMA/SALIVA/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pmol/mL)	22.6	68.7	116	21.7	70.4	119
Standard deviation	1.37	2.55	2.54	2.2	6.0	7.3
CV (%)	6.0	3.7	2.2	10.3	8.6	6.1

CELL LYSATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pmol/mL)	18.5	56.9	96.1	17.0	51.8	89.2
Standard deviation	0.89	1.74	2.73	1.3	3.5	6.9
CV (%)	4.8	3.1	2.8	7.4	6.8	7.7

RECOVERY

The recovery of cAMP spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range %
Cell culture media* (n=4)	104	86-119%
Cell Lysis Buffer 5 (n=1)	91	87-94%
Serum* (n=4)	103	97-114%
EDTA plasma* (n=4)	105	92-120%
Heparin plasma* (n=4)	98	85-111%
Saliva* (n=4)	97	80-117%
Urine* (n=6)	100	91-119%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of cAMP were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=4)	Cell Lysis Buffer 5 (n=1)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Saliva* (n=4)	Urine* (n=4)
1:2	Average % of Expected	102	100	93	91	98	103	96
	Range (%)	95-112	——	85-100	88-95	92-103	89-114	95-97
1:4	Average % of Expected	96	101	95	95	99	98	95
	Range (%)	92-102	——	89-99	90-97	95-105	88-106	91-97
1:8	Average % of Expected	91	97	94	96	99	90	83
	Range (%)	83-99	——	91-101	92-99	91-107	85-96	75-90
1:16	Average % of Expected	91	98	86	92	94	87	——
	Range (%)	84-98	——	79-92	82-99	88-106	82-92	——

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Ninety assays were evaluated and the minimum detectable dose (MDD) of cAMP ranged from 0.42-8.57 pmol/mL. The mean MDD was 1.50 pmol/mL.

The MDD was determined by subtracting two standard deviations from the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of cAMP in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pmol/mL)	Range (pmol/mL)	Standard Deviation (pmol/mL)
Serum* (n=34)	80.3	35.8-204	37.7
EDTA plasma* (n=34)	82.9	44.6-208	35.5
Heparin plasma* (n=34)	64.0	27.2-166	29.3
Urine* (n=27)	5299	767-15,486	4068

*Samples were diluted prior to assay as directed in the Sample Preparation section.

Cell Culture Supernates - A549 human lung carcinoma, HepG2 human hepatocellular carcinoma, and KATO-III human gastric carcinoma cell culture supernates, as well as stimulated and unstimulated PBLs, were assayed for levels of cAMP. No detectable levels were observed.

Cell Lysates - A549 human lung carcinoma cells were grown in Kaighn's F-12 media supplemented with 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, and 10% fetal bovine serum for 4 days at 37 °C, 95% humidity, and 5% CO_2. Cells were lysed, assayed for levels of cAMP, and measured 12.7 pmol/mL.

Saliva - Saliva samples from apparently healthy volunteers were diluted prior to assay and evaluated for the presence of cAMP. No medical histories were available for the donors used in this study. Twenty-one samples ranged from 13.4-46.0 pmol/mL with a detectable mean of 25.5 pmol/mL. One sample measured below the low standard, 3.75 pmol/mL.

SPECIFICITY

The factors listed below were prepared at 24,000 pmol/mL in calibrator diluent and Cell Lysis Buffer 5 (diluted 1:5) and assayed for cross-reactivity. Preparations of the following factors at 24,000 pmol/mL in a mid-range cAMP standard were assayed for interference. No significant cross-reactivity or interference was observed.

AMP	CTP	GMP	UMP
ATP	GTP	cGMP	

Phosphodiesterase inhibitors used in cell culture media were tested in this assay by diluting them in RPMI with 10% fetal bovine serum. Denbufylline, Ro-20-1724, rolipram, pentoxifylline, IBMX, and etazolate hydrochloride did not cross-react or interfere in this assay.

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