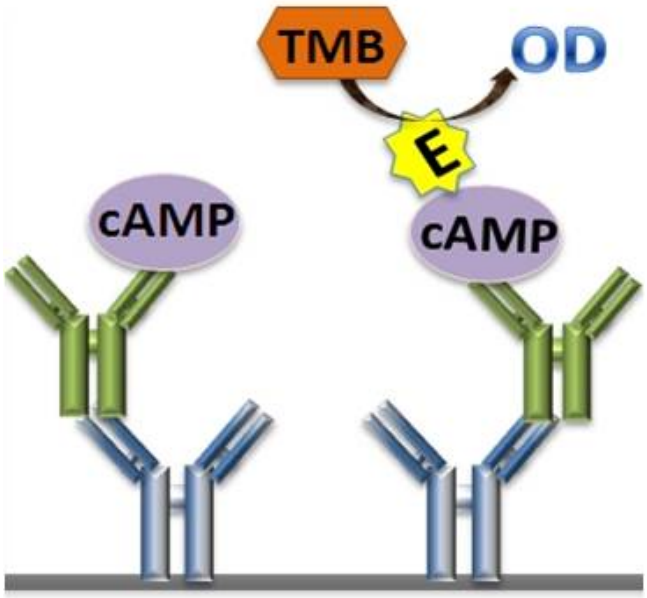
cAMP ELISA Detection Kit

Cat. No. L00460 Version 02242014



The operator should read technical manual carefully before using this product.

For research use only. Not for diagnostic use.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

Contents

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

I. DESCRIPTION

Adenosine 3', 5' cyclic monophosphate (cAMP) is an important secondary messenger in signal transduction pathways that follows a number of extracellular signals. The G-Protein Coupled Receptors (GPCRs) are critical cell surface receptors that can be activated by different ligands, such as neurotransmitters, hormones and ions. The binding of the ligand to its receptor activates the effector adenylyl cyclase generating cAMP product. cAMP activates or inhibits various enzymes by promoting their phosphorylation or dephosphorylation. Its concentration is converted from adenosine triphosphate (ATP) via adenylyl cyclases (AC), and is inactivated by hydrolysis to 5´-AMP by the actions of phosphodiesterases.

GenScript cAMP ELISA Detection Kit is a competition enzyme-linked immunoassay which can be used for quantitative detection of cAMP in samples such as serum, plasma, saliva, cell culture supernatant, and urine. The anti-IgG Capture Plate is pre-coated with a fixed amount of Goat anti-mouse IgG to capture Mouse Anti-cAMP Monoclonal Antibody. When free cAMP or specimen and HRP-cAMP are added to the well, they compete in the solution to interact with the cAMP antibody captured on the plate. Other unbound molecules are removed by the wash step. The cAMP-HRP reacts with TMB substrate to develop a blue product in the solution. The reaction is stopped by adding stop solution and the color turns yellow which can be read at 450 nm by a Microtiter plate reader. Because the concentration of cAMP-HRP is held constant in the assay, the intensity of color is inversely proportional to the cAMP concentration in the sample and standards. Using the standard curve, the cAMP amount present in the unknown samples can be calculated by transforming its absorbance value.

II. KEY FEATURES

Features	Specifications
Sensitivity	0.61 pmol/ml
Detection Range	1~243 pmol/ml
Test Samples	Serum, plasma, saliva, cell culture supernatant, and urine
Conveniency	All reagents and buffers for cAMP test are provided Complete the test within 3.5 hours
Cross Reactivity	No significant cross-reactivity of similar compounds was found (see Cross-reactivity)

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

III. KIT CONTENTS

- The cAMP Standard Stock can be used to prepare cAMP Standards with Assay Buffer A or cell culture medium as an alternative.
- The kit provides all reagents and buffers for cAMP detection.

Component	Quantity	Part. No
Anti-IgG Capture Plate	1 plate (8 wells x 12 strips)	460-80
Anti-cAMP mAb	12 ml	460-20
HRP-cAMP	6 ml	460-30
cAMP Standards (0, 1, 3, 9, 27, 81, 243 pmol/ml)	1.5 ml	460-11, 12, 13, 14, 15, 16,17
cAMP Standard Stock (10 nmol/ml)	500 µl	460-10
Assay Buffer A	60 ml	460-60
Assay Buffer B	1 ml	460-90
20 × Wash Solution	40 ml	460-70
TMB Substrate	12 ml	460-40
Stop Solution	6 ml	460-50
Plate Sealer	2 pieces	N/A
User Manual	1 copy	N/A

IV. STORAGE

The unopened kit is stable for at least 12 months if stored at 2-8 °C, and the opened kit is stable for up to 2 weeks at 2-8 °C. Do not freeze the kit.

V. REAGENTS/EQUIPMENT BUT NOT SUPPLIED

Microtiter plate reader capable of measuring absorbance at 450 nm
Automated microplate washer to wash the plate
Deionized or distilled water to dilute 20 x Wash Solution
Graduated cylinder to prepare Wash Solution
Plastic container to store Wash Solution
Tubes to aliquot and dilute samples
Precision pipettes to deliver 10 µl, 100 µl, 200 µl and 1000 µl content
10 µl, 100 µl, 200 µl and 1000 µl pipette tips
Multichannel pipettor
Disposable reagent reservoir
Paper towel

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

Laboratory timer

Refrigerator to store samples and kit components

VI. PROTOCOL

- All reagents in the kit and test samples should be equilibrated to room temperature before test.
- Preliminary experiment should be performed to optimize the sample dilution.

1. Sample Preparation

- Handle serum and plasma samples in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of blood-borne infection.
- Assay Buffer A is used for serum, plasma, saliva, and urine sample dilution.
- Free cell culture medium is used for standard curve preparation for cell culture sample and cell culture sample dilution.

Serum: Use a blood separator tube and allow the sample to clot for 30 minutes. Centrifuge for 10 minutes at 1000 x g. Run the assay immediately, otherwise aliquot and store the samples below -20 °C. Avoid repeated freeze-thaw cycles. Serum samples usually require a 20-fold dilution.

Plasma: Treat the blood with heparin as an anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes for plasma collection. Run the assay immediately. Otherwise aliquot and store the samples below -20 °C. Avoid repeated freeze-thaw cycles. Plasma samples require a 10-fold dilution.

- Blood samples with citrate and EDTA treatment are not recommended for the assay.

Cell culture: Centrifuge the sample to remove the particulate materials. Run the assay immediately, otherwise aliquot and store the samples below -20 °C. Avoid repeated freeze-thaw cycles. Cell culture samples usually require a 2-fold dilution.

- For cell culture sample test, it is recommended to use free medium to prepare standard curve with cAMP Standard Stock.

Saliva: Collect saliva using a collection device into a sterile container. Run the assay immediately, otherwise aliquot and store the samples below -20 °C. Avoid repeated freeze-thaw cycles. Saliva samples usually require a 5-fold dilution.

Urine: Collect the urine into a sterile container. Centrifuge for 10 minutes at 1000 x g. Run the assay immediately, otherwise aliquot and store the samples below -20 °C. Avoid repeated freeze-thaw cycles. Urine samples usually require a 100-fold dilution.

2. Reagent Preparation

- If any precipitate is found in the 20 × Wash Solution, incubate the bottle in water bath (up to 50 °C) with occasional mixing until all the precipitate is dissolved.

1 x Wash Solution: Dilute *20 × Wash Solution* by 1:19 v/v with deionized or distilled water. For example, dilute 40 ml of *20 × Wash Solution* with 760 ml of deionized or distilled water to make 800 ml of 1 × *Wash Solution*. Store at 2-8 °C.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

cAMP Standards Preparation

- The kit provides a set of cAMP standards for serum, plasma, saliva, and urine sample test.
- For culture sample test, free culture medium is recommended to prepare cAMP standards.
- For other sample test, Assay Buffer A is recommended to prepare cAMP standards.
- Allow cAMP Standard Control to sit for a minimum of 15 minutes at room temperature with gentle agitation prior to making dilution.

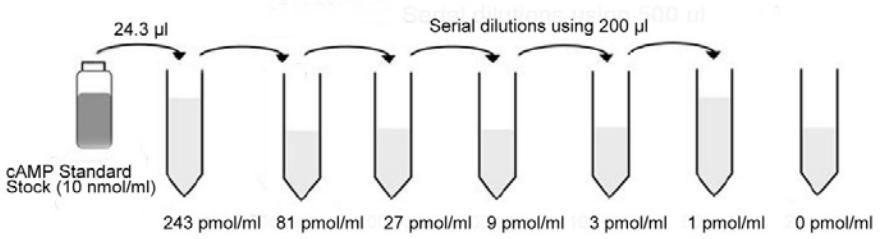
2.1 Label seven clear 1.5 ml Eppendorf tubes with '243 pmol/ml', '81 pmol/ml', '27 pmol/ml', '9 pmol/ml', '3 pmol/ml', '1 pmol/ml' and '0 pmol/ml'.

2.2 Pipette 24.3 µl of cAMP Standard and 975.7 µl of Assay Buffer A or cell culture medium into the tube labeled with '243 pmol/ml' and vortex it.

2.3 Pipette 400 µl of Assay Buffer A or cell culture medium into the rest of the empty tubes.

2.4 Pipette 200 µl of 243 pmol/ml of cAMP solution to the tube labeled with '81 pmol/ml' and vortex it to make the standard 81 pmol/ml.

2.5 Similarly, prepare the rest of the standard series (81, 27, 9, 3, 1 pmol/ml).



Anti-IgG Capture Plate Preparation

- It is recommended that all cAMP standards and samples be prepared in duplicate.
- Count the strips for the assay and make sure the strips are tightly snapped in the plate frame.
- Leave the unused strips in the foil pouch and store at 2-8 °C. The strips must be stored in the closed foil pouch to prevent moisture because the moisture can damage the Anti-IgG Capture Plate.

3. Test Procedure

Anti-cAMP mAb Incubation

3.1 Add 100 µl of Anti-cAMP mAb to all wells.

3.2 Cover the plate with *Plate Sealer* and incubate at 25 °C for one hour.

3.3 Remove the Plate Sealer and wash the plate with 260 µl of *1 x Wash Solution* for four times.

3.4 Pat the plate on paper towel to remove residual liquid in the wells after wash step.

cAMP/HRP-cAMP Incubation

- For cell culture sample test, free cell culture medium is added to the non-specific binding (NSB) wells instead of Assay Buffer A.

3.5 Add 100 µl of Assay Buffer A to the NSB wells and 100 µl of a set of cAMP standards and samples to the remaining wells separately.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

3.6 Add 50 µl of Assay Buffer B to the NSB wells and 50 µl of cAMP-HRP to the remaining wells.

3.7 Cover the plate with *Plate Sealer* and incubate at 4 °C for 2 hours.

3.8 Remove the Plate Sealer and wash the plate with 260 µl of *1 x Wash Solution* for four times.

3.9 Pat the plate on paper towel to remove residual liquid in the wells after wash step.

Substrate Reaction and Absorbance Measurement

3.10 Add 100 µl of *TMB Substrate* to all the wells and incubate at 25 °C for 15-20 minutes and protect it from light.

3.11 Add 50 µl of *Stop Solution* to all the wells to stop the enzyme reaction.

3.12 Read the plate on Microtiter plate reader at 450 nm.

4. Calculation of Data

- To ensure test stability, read the plate at 450 nm immediately after adding *Stop Solution*.
- If the sample is diluted, multiply the interpolated value by the dilution factor to calculate the amount of cAMP concentration in the sample.

4.1 Average the optical densities (ODs) for each set of replicate wells.

4.2 Subtract the average NSB OD from the average OD for each standard and sample to calculate adjusted average OD.

Adjusted average OD = Average OD – Average NSB OD

4.3 Calculate the B/B_0 for each standard and sample using the following relationship

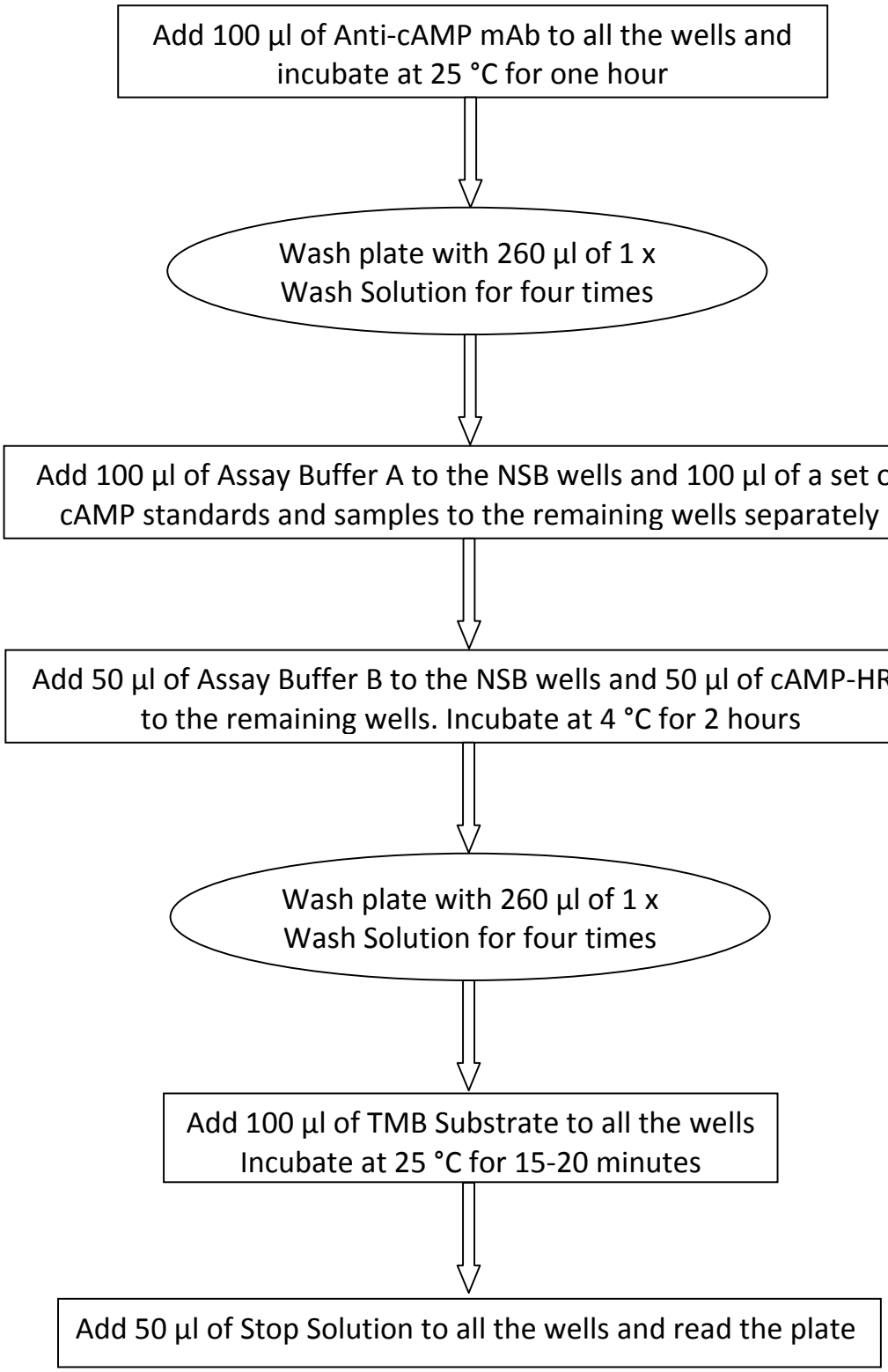
$$B/B_0\ (\%) = \frac{\text{Standards or sample adjusted average OD}}{\text{Zero standard adjusted average OD}} \times 100$$

4.4 Generate a standard curve by plotting the B/B_0 on the vertical (Y) axis versus the cAMP standard concentration on the horizontal (X) axis.

4.5 The amount of cAMP concentration in sample is determined by extrapolating its B/B_0 to the standard curve.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

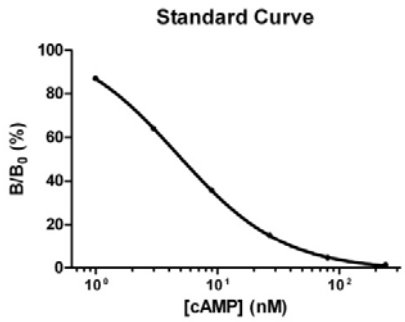
VII. ASSAY PROCEDUR SUMMARY



860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

VIII. TYPICAL ASSAY DATA

The standard curve below was provided for demonstration only. Operator should set up standard curve to precisely determine cAMP each time.



cAMP Standard (pmol/ml)	OD_{450}				%B/B$_O$
	Duplicate 1	Duplicate 2	Average	Adjusted Average	
NSB	0.051	0.051	0.051	-	-
0	1.446	1.447	1.447	1.396	-
1	1.271	1.251	1.261	1.210	86.68%
3	0.946	0.935	0.941	0.890	63.72%
9	0.557	0.537	0.547	0.496	35.53%
27	0.247	0.267	0.257	0.206	14.76%
81	0.114	0.112	0.113	0.062	4.44%
243	0.066	0.067	0.067	0.016	1.11%

IX. PRECISION

Intra-assay: Three different known levels of cAMP were spiked into Assay Buffer A as test samples. All samples were tested 20 times on the same plate to evaluate Intra-assay precision of the kit.

Inter-assay: Three different known levels of cAMP were spiked into Assay Buffer A as test samples. All samples were tested in 20 separate assays to evaluate Inter-assay precision of the kit.

Intra-assay				Inter-assay			
# of replicates	Mean (pmol/ml)	SD	CV%	# of replicates	Mean (pmol/ml)	SD	CV%
20	2.33	0.21	9.0	20	2.57	0.26	10.1
20	4.59	0.35	7.6	20	4.99	0.53	10.6
20	8.89	0.45	5.1	20	10.54	1.01	9.6

X. SENSITIVITY

The minimum detectable dose (MDD) of the assay was between 0.33-0.90 pmol/ml. The mean MDD was 0.61 pmol/ml.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

XI. RECOVERY

Different known levels of cAMP were spiked into various samples to prepare sample matrices. The matrices were assayed with the kit to determine the spiked cAMP concentrations. The percentage was determined by dividing the calculated cAMP concentraion of the spiked cAMP by the corresponding practically spiked cAMP level for each sample to evaluate the assay recovery.

Sample	Mean Recovery (%)	Range (%)
Serum (n=5)	95	84-104
Heprin Plasma (n=5)	102	93-113
Urine (n=5)	101	86-114
Saliva (n=5)	103	92-119
Cell Culture Supernatant (n=5)	95	84-104

XII. LINEARITY

High known levels of cAMP were spiked into various samples and then the spiked samples were serially diluted with Assay Buffer A to prepare sample matrices. The matrices were assayed with the kit to determine the spiked cAMP concentrations of all diluted samples. The percentage was determined by dividing the calculated cAMP concentration of the spiked cAMP by the corresponding practically spiked cAMP level for each sample to evaluate the assay linearity.

Dilution		Serum (n=5)	Heparin plasma (n=5)	Urine (n=5)	Saliva (n=5)
1:2	Average % of Expected	95	105	99	103
	Range (%)	87-113	96-111	95-106	94-113
1:4	Average % of Expected	96	102	100	98
	Range (%)	85-118	88-109	98-103	90-103
1:8	Average % of Expected	92	94	100	94
	Range (%)	87-99	91-99	97-104	84-101

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

XIII. CROSS-REACTIVITY

A variety of compounds with similar structure were tested in the assay to evaluate cross-reactivity. A series of compound concentrations were spiked in Assay Buffer A as test samples. These samples were then measured in the assay and the compound concentration, which gave B/B_0 at 50%, was calculated. The cross-reactivity of the kit was defined as the ratio of cAMP concentration with B/B_0 at 50% to compound concentration with B/B_0 at 50% in the assay. No significant cross-reactivity of the tested compounds was observed in the assay which indicated the below compounds in the samples had no interference on cAMP determination.

Compound	Cross reactivity
ATP	0.4717%
cIMP	0.3060%
cGMP	0.1442%
cTMP	0.0091%
GTP	0.0001%
CTP	<0.0001%
ADP	<0.0001%
AMP	<0.0001%
GDP	<0.0001%
GMP	<0.0001%
UMP	<0.0001%

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

XIV. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Poor Standard Curve	Improper preparation of standards	Prepare new standards as the manual describes
	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Pipetting error	Check pipette calibration and repeat assay
	Components are used from other lots or sources	Never substitute any components from another kit
	Components are not brought to room temperature prior to assay	Repeat assay with components that have been equilibrated to room temperature
	Incubation steps are performed at wrong temperatures	Perform incubation step as the manual describes
Weak/No Signal	TMB substrate are not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	TMB substrate is contaminated	Use new TMB substrate
	Did not add the proper volumes of reagents	Repeat assay with the required volumes in manual
	Did not incubate the plate for proper time or temperature	Follow the manual to repeat assay
	Did not read the plate immediately after stop solution was added	Read the plate within 30 minutes after adding stop solution
High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	TMB substrate is contaminated	Use new TMB substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with plate sealer in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay
	TMB substrate is exposed to light	Use new TMB substrate

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

XV. RELATED PRODUCTS

- THE™ cAMP Antibody, mAb, Mouse A01509-100
- cAMP Antibody, pAb, Rabbit A00614-40
- cAMP-HRP M01059
- THE™ cGMP Antibody, mAb, Mouse A01508-100
- cGMP Antibody, pAb, Rabbit A00615
- cGMP-HRP M01058
- cGMP ELISA Detection Kit L00461
- THE™ ADP Antibody, mAb, Mouse A01799-100
- ADP Antibody, pAb, Rabbit A01316

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

XVI. Plate Layout

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes:

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: order @genscript.com Web: www.genscript.com

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes:

GenScript US
860 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: product@genscript.com
Web: http://www.genscript.com

For Research Use Only.