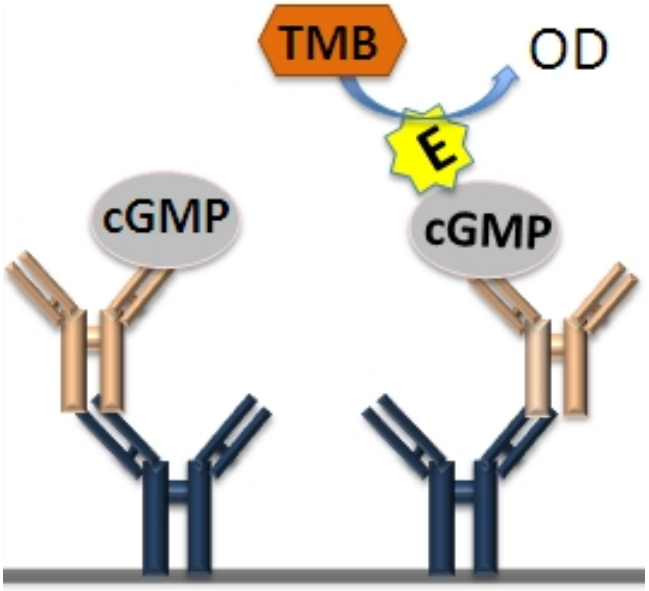
cGMP ELISA Detection Kit

Cat. No. L00461 Version 02242014



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

For research use only. Not for diagnostic use.

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Contents

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I. DESCRIPTION

Guanosine 3', 5'-cyclic monophosphate (cyclic GMP; cGMP) is an important secondary messenger in signal transduction pathways. It is a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis. cGMP is a cyclic nucleotide derived from guanosine triphosphate (GTP). The cellular production of cGMP is mediated by Guanylate cyclase (GC). It activates the cGMP-dependent protein kinases which in turn phosphorylate several downstream protein targets. cGMP has an effect on the regulation of cAMP levels by activating or inhibiting specific phosphodiesterases (PDEs).

GenScript cGMP ELISA Detection Kit is a competition enzyme-linked immunoassay which can be used for quantitative detection of cGMP in samples such as serum, plasma, saliva, cell culture supernatant, and urine. The anti-IgG Capture Plate is pre-coated with fixed amount of Goat anti-rabbit IgG to capture Rabbit Anti-cGMP Polyclonal Antibody. When free cGMP or specimen and HRP-cGMP conjugate are added to the well, they compete in the solution to interact with the cGMP antibody captured on the plate. Other unbound molecules are removed by a wash step. The cGMP-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. Becuase the concentration of cGMP-HRP is held constant in the assay, the intensity of color is inversely proportional to the cGMP concentration in the sample and standards. Using the standard curve, the amount of cGMP present in the unknown samples can be calculated by transforming its absorbance value.

II. KEY FEATURES

Features	Specifications
Sensitivity	0.11 pmol/ml
Detection Range	0.3-66.7 pmol/ml
Test Samples	Serum, plasma, saliva, cell culture supernatant, and urine
Conveniency	All reagents and buffers for test are provided Complete the test within 2.5 hours
Cross-reactivity	No significant cross-reactivity of similar compounds was found (See Cross-reactivity)

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III. KIT CONTENTS

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

Component	Quantity	Part. No
Anti-IgG Capture Plate	1 plate (8 wells x 12 strips)	461-80
Anti-cGMP pAb	12 ml	461-20
HRP-cGMP	6 ml	461-30
cGMP Standards (0, 0.3, 0.8, 2.5, 7.4, 22.2, 66.7 pmol/ml)	1.5 ml	461-11,12,13,14,15,16,17
cGMP Standard Stock (10 nmol/ml)	500 µl	461-10
Assay Buffer A	60 ml	461-60
Assay Buffer B	1 ml	461-90
20 × Wash Solution	40 ml	461-70
TMB Substrate	12 ml	461-40
Stop Solution	6 ml	461-50
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

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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, cell culture, and urine 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 10-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 usually 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.

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 20-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.

cGMP Standards Preparation

- Allow cGMP Standard Control to sit for a minimum of 15 minutes at room temperature with gentle agitation prior to making dilution.

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- This step is an alternative when cGMP Standards have run out.

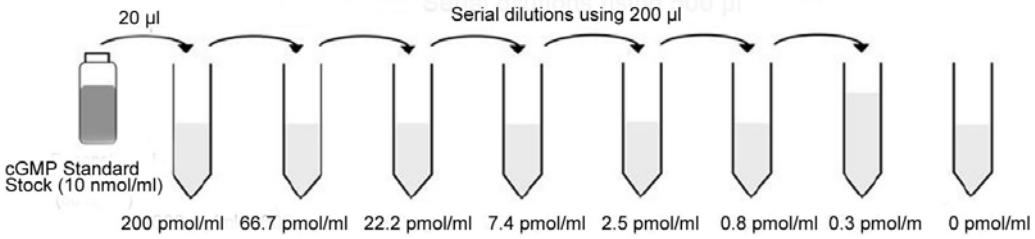
2.1 Label eight 1.5 ml Eppendorf tubes with '200 pmol/ml', '66.7 pmol/ml', '22.2 pmol/ml', '7.4 pmol/ml', '2.5 pmol/ml' , '0.8 pmol/ml' , '0.3 pmol/ml' , '0 pmol/ml'.

2.2 Pipette 20 µl of cGMP Standard Stock (10 nmol/ml) and 980 µl of Assay Buffer A to the tube labeled with '200 pmol/ml' and vortex it.

2.3 Pipette 400 µl of Assay Buffer A to the rest of the empty tubes.

2.4 Pipette 200 µl of 200 pmol/ml of cGMP solution to the tube labeled with '66.7 pmol/ml' and vortex it to make the standard 66.7 pmol/ml.

2.5 Similarly, prepare the rest of the standard series (22.2, 7.4, 2.5, 0.8, 0.3 pmol/ml).



Anti-IgG Capture Plate Preparation

- It is recommended that all cGMP 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-cGMP pAb Incubation

3.1 Add 100 µl of Anti-cGMP pAb to all wells.

3.2 Cover the plate with the *Plate Sealer* and incubate at 25 °C for 45 minutes.

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.

cGMP/HRP-cGMP Incubation

3.5 Add 100 µl of Assay Buffer A to the non-specific binding (NSB) wells and 100 µl of a set of cGMP standards and samples to the remaining wells separately.

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

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

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.

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Substrate Reaction and Absorbance Measurement

- To ensure test stability, read the plate at 450 nm immediately after adding Stop Solution.

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

- If the sample is diluted, multiply the interpolated value by the dilution factor to calculate the amount of cGMP concentration in the sample.

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

4.1 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

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

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

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

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

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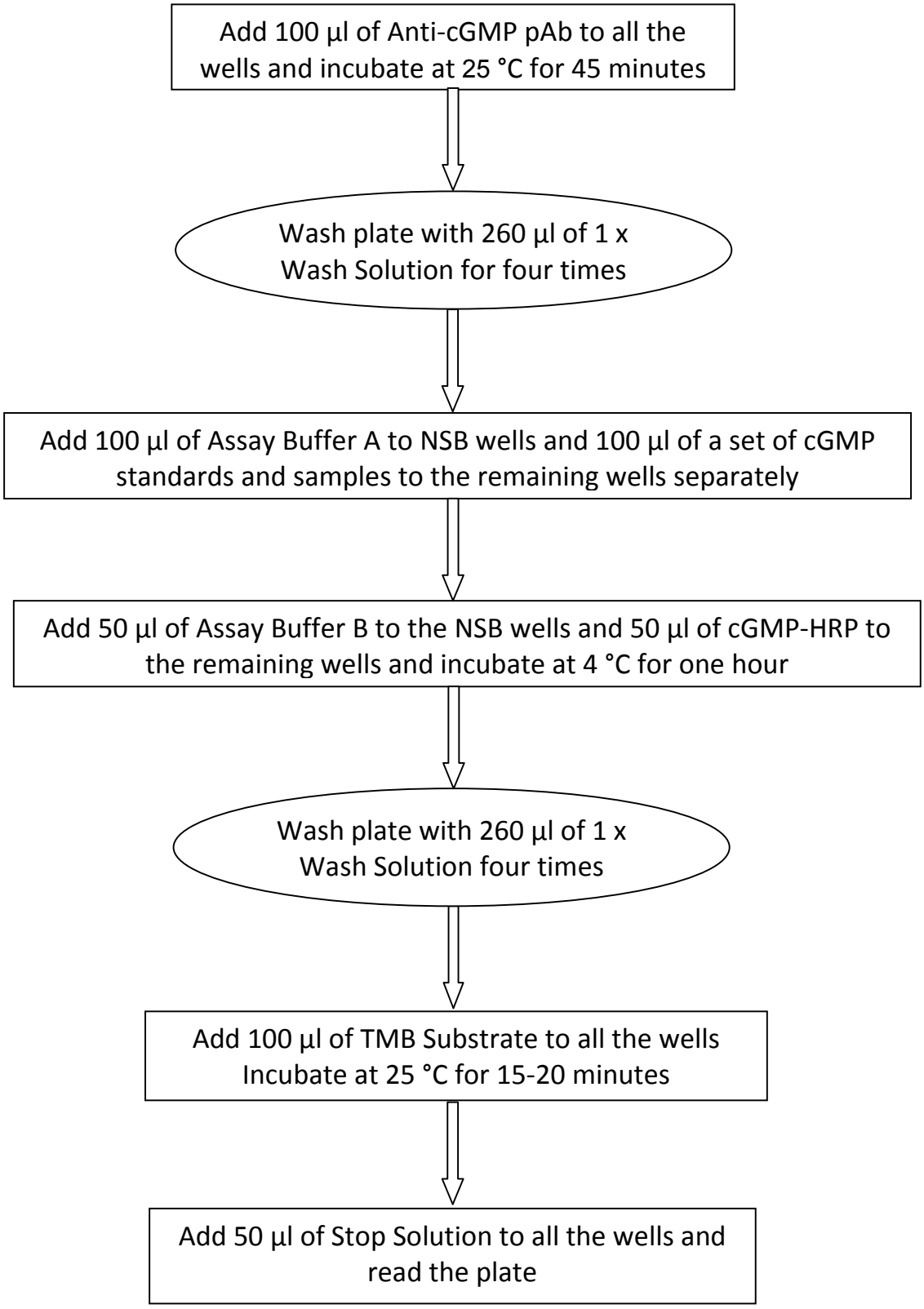
VII. ASSAY PROCEDUR SUMMARY



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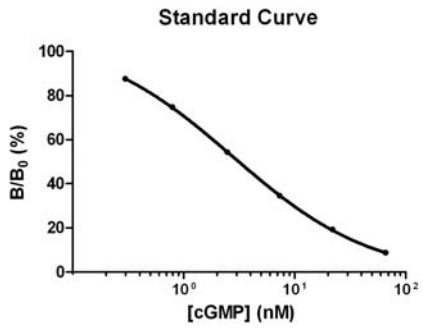
VIII. TYPICAL ASSAY DATA

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



cGMP Standard (pmol/ml)	OD_{450}				%B/B$_O$
	Duplicate 1	Duplicate 2	Average	Adjusted Average	
NSB	0.048	0.048	0.048	-	-
0	1.038	1.040	1.039	0.991	-
0.3	0.925	0.926	0.926	0.878	88.55%
0.8	0.769	0.797	0.783	0.735	74.17%
2.5	0.612	0.643	0.628	0.580	58.48%
7.4	0.392	0.446	0.419	0.371	37.44%
22.2	0.250	0.261	0.256	0.208	20.94%
66.7	0.157	0.146	0.152	0.104	10.44%

IX. PRECISION

Intra-assay: Three different known levels of cGMP 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 cGMP 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	2.56	0.21	8.2	20	2.65	0.21	7.9
20	5.11	0.37	7.2	20	5.24	0.45	8.6
20	11.15	0.69	6.2	20	10.32	0.90	8.7

X. SENSITIVITY

The minimum detectable dose (MDD) of the assay was between 0.04-0.18 pmol/ml. The mean MDD was 0.11 pmol/ml.

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XI. RECOVERY

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

Sample	Mean Recovery (%)	Range (%)
Serum (n=5)	102	87-118
Heprin Plasma (n=5)	99	86-108
Urine (n=5)	93	83-109
Saliva (n=5)	103	93-117
Cell Culture Supernatant (n=5)	103	82-111

XII. LINEARITY

High known levels of cGMP 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 cGMP concentrations. The percentage was determined by dividing the calculated cGMP concentration of the spiked cGMP by the corresponding practically spiked cGMP level for each sample to evaluate the assay linearity.

Dilution		Serum (n=5)	Heparin Plasma (n=5)	Urine (n=5)	Saliva (n=5)	Cell Culture (n=5)
1:2	Average % of Expected	105	95	104	100	99
	Range (%)	85-113	93-103	90-110	90-109	94-102
1:4	Average % of Expected	91	94	98	108	100
	Range (%)	88-96	86-111	98-99	98-117	94-105
1:8	Average % of Expected	97	102	100	101	100
	Range (%)	84-114	97-108	97-104	94-110	96-105

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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 prepared in Assay Buffer A as test samples. These samples were then measured in the assay and the compound concentration, which generated B/B_0 at 50%, was calculated. The cross-reactivity of the kit was defined as the ratio of cGMP concentration with B/B_0 at 50% to the 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 cGMP determination.

Compound	Cross Reactivity
cIMP	0.0110%
cTMP	0.0017%
cAMP	0.0008%
GTP	0.0005%
GMP	0.0002%
GDP	<0.0001%
ATP	<0.0001%
ADP	<0.0001%
AMP	<0.0001%
UMP	<0.0001%
CTP	<0.0001%

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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

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XV. RELATED PRODUCTS

- THE™ cAMP Antibody, mAb, Mouse A01509-100
- cAMP Antibody, pAb, Rabbit A00614-40
- cAMP ELISA Detection Kit L00460
- cAMP-HRP M01059
- THE™ cGMP Antibody, mAb, Mouse A01508-100
- cGMP Antibody, pAb, Rabbit A00615
- cGMP-HRP M01058
- THE™ ADP Antibody, mAb, Mouse A01799-100
- ADP Antibody, pAb, Rabbit A01316

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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:

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