Product Manual

cGMP ELISA Kit (Colorimetric)

Catalog Numbers

STA-505	96 assays
STA-505-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Guanosine 3',5'-cyclic monophosphate (cGMP) is a multi-functional second messenger involved in various cellular activities in many cell and tissue types. It is converted from guanosine triphosphate (GTP) via guanylyl cyclases (GC). It has been shown that the level of cGMP is typically 10-100 fold lower than cAMP in most tissues. cGMP primarily affects cellular activities through four different pathways. These include cGMP-dependent Protein Kinases (PKG/GK), cyclic nucleotide-gated (CNG) channels, cAMP-dependent Protein Kinase (PKA), and Phosphodiesterases.

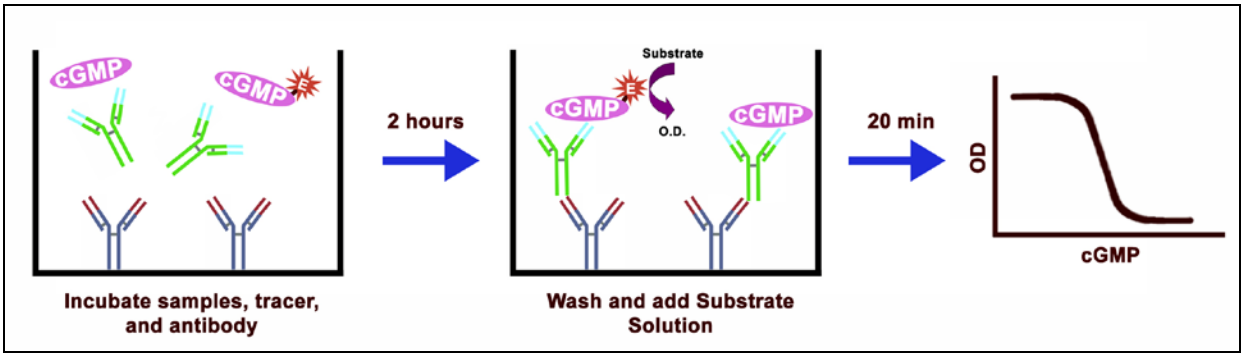
PKGs (PKG I and PKG II) are serine/threonine kinases activated by cGMP. PKG I has several putative targets, many of which are involved in the regulation of smooth muscle cell (SMC) contractility. Other cGMP/PKG I substrates that affect contractility include the myosin-binding subunit (MBS) of MLC Phosphatase and the small GTPase Rho. In addition to its role in smooth muscle relaxation, cGMP/PKG I may also regulate cell survival, proliferation, axon guidance, synaptic plasticity, inflammation, and angiogenesis.

Cell Biolabs' cGMP ELISA Kit is a competitive enzyme immunoassay designed to measure cGMP in cell culture supernatants, plasma, serum, saliva, urine, and cell lysates. The kit selectively measures cGMP levels without any significant cross reactivities to other nucleotides or cyclic nucleotides. Samples containing low cGMP levels may be acetylated (reagents provided) for increased sensitivity. Under non-acetylated conditions, the kit has a detection range of 1 to 1000 pmol/mL cGMP; however, under acetylated conditions, the sensitivity is enhanced (approx 50X) to a detection range of 100-6250 fmol/mL.

Assay Principle

An anti-Rabbit IgG polyclonal coating antibody is adsorbed onto a microtiter plate. Cyclic GMP present in the sample or standard competes with Peroxidase cGMP Tracer for plate binding, in the presence of Rabbit Anti-cGMP Polyclonal Antibody.

Following incubation and wash steps, any Peroxidase cGMP Tracer bound to the plate is detected with addition of Substrate Solution. The colored product formed in inversely proportional to the amount of cGMP present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from cGMP Standard and sample concentration is then determined.



Related Products

1. STA-500: cAMP ELISA Kit (Colorimetric)
2. STA-501: cAMP ELISA Kit (Chemiluminescent)
3. STA-506: cGMP ELISA Kit (Chemiluminescent)

Kit Components

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250001): One strip well 96-well plate.
2. cGMP Standard (Part No. 250501): One 200 µL vial provided at 10 mM.
3. Rabbit Anti-cGMP Polyclonal Antibody (Part No. 250502): One 15 µL vial.
4. Peroxidase cGMP Tracer Conjugate (Part No. 250503): One 30 µL vial.
5. Assay Diluent (Part No. 250005): One 25 mL bottle.
6. Lysis Buffer (Part No. 250006): One 50 mL bottle.
7. 10X Wash Buffer (Part No. 250007): One 50 mL bottle.
8. Triethylamine (Part No. 250008): One 2 mL amber bottle.
9. Acetic Anhydride (Part No. 250009): One 1 mL amber bottle.
10. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
11. Stop Solution (Part. No. 310808): One 12 mL bottle.

Materials Not Supplied

1. Orbital plate shaker
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
6. Glass or polypropylene tubes for acetylated samples and standards

Storage

Store kit components at 4ºC. For longer term use, store the Rabbit Anti-cGMP Polyclonal Antibody at -20ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Rabbit Anti-cGMP Polyclonal Antibody: Immediately before use dilute the Rabbit Anti-cGMP Antibody 1:500 with Assay Diluent. Do not store diluted solutions.

- Peroxidase cGMP Tracer Conjugate: Immediately before use dilute the Peroxidase cGMP Tracer Conjugate 1:100 with Assay Diluent. Do not store diluted solutions.
- Acetylation Reagent: Preparation of the Acetylation Reagent should be done in glass tubes and in a fume hood. The Acetylation Reagent is made by mixing Acetic Anhydride with Triethylamine at a 1:2 ratio (example: 0.5 mL Acetic Anhydride + 1 mL Triethylamine). Use the reagent within 60 minutes of preparation.

  Caution: The components of this reagent are known to be caustic, corrosive, flammable, and lachrymators. Use appropriate protection when handling.

Preparation of cGMP Standards (Non-Acetylated Version)

1. Thaw the cGMP Standard at room temperature and mix thoroughly by pipetting (cGMP can precipitate when frozen but will redissolve when mixed well). Freshly prepare a dilution series of cGMP Standard in the concentration range of 1 mM – 1 nM by diluting the cGMP Standard in Lysis Buffer (Table 1).

Standard Tubes	cGMP Standard (µL)	Lysis Buffer (µL)	cGMP Concentration
1	40	360	1 mM
2	20 of Tube #1	180	100 µM
3	20 of Tube #2	180	10 µM
4	20 of Tube #3	180	1 µM
5	20 of Tube #4	180	100 nM
6	20 of Tube #5	180	10 nM
7	20 of Tube #6	180	1 nM
8	0	180	0

Table 1. Preparation of Non-Acetylated cGMP Standard Curve

Preparation of Samples (Non-Acetylated Version)

- Urine, Serum, Plasma and Culture Medium Samples: Urine, serum and plasma may be tested directly or diluted with Lysis Buffer. Culture medium can also be tested with dilutions in Lysis Buffer.
- Cell Samples: Aspirate medium. Add 1 ml of Lysis Buffer for every 35 cm^2 of surface area. Incubate at 4 °C for 20 minutes. Scrape cells off the surface with a cell scraper. Dissociate sample by pipetting up and down until suspension is homogeneous. Transfer to a centrifuge tube and centrifuge at top speed for 10 min. The supernatant can be assayed directly. Protein concentration >1 mg/ml is recommended for reproducible results.
- Tissue Samples: Cyclic nucleotides may be metabolized quickly in tissue, so it is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen). Weigh the frozen tissue and add 5-10 µL of Lysis Buffer per mg of tissue. Homogenize the sample on ice using a Polytron-type homogenizer. Spin at top speed for 5 min and collect the supernatant. The supernatant may be assayed directly.

Preparation of cGMP Standards (Acetylated Version)

Note: Samples containing low cGMP levels may be acetylated for increased sensitivity (approx 50-fold), although overall assay values will be lowered 2-3 fold.

1. Thaw the cGMP Standard at room temperature and mix thoroughly by pipetting (cGMP can precipitate when frozen but will redissolve when mixed well). In glass or polypropylene tubes, freshly prepare a dilution series of cGMP Standard in the concentration range of 100 nM – 24 pM by diluting the cGMP Standard in Lysis Buffer (Table 2).

   Note: The kit cGMP Standard, provided at 10 mM, must first be aggressively diluted to achieve the desired range. A series of dilutions are suggested (denoted Stock A and B). Stock A and B are <u>not</u> to be included in the standard curve; only tubes 1-8 should be transferred.

Standard Tubes	cGMP Standard (µL)	Lysis Buffer (µL)	Final cGMP Concentration
Stock A	10 of cGMP Standard (10 mM)	990	100 uM
Stock B	10 of Stock A	990	1 uM
1	40 of Stock B	360	100 nM
2	100 of Tube #1	300	25 nM
3	100 of Tube #2	300	6.25 nM
4	100 of Tube #3	300	1.56 nM
5	100 of Tube #4	300	391 pM
6	100 of Tube #5	300	98 pM
7	100 of Tube #6	300	24 pM
8	0	300	0

Table 2. Preparation of Acetylated cGMP Standard Curve

2. In the hood, transfer 200 µL of tubes 1-8 to new tubes and acetylate each by adding 10 µL of Acetylation Reagent (see Preparation of Reagents). Mix well and use within 30 minutes.

Preparation of Samples (Acetylated Version)

Note: Samples containing low cGMP levels may be acetylated for increased sensitivity (approx 50-fold), although overall assay values will be lowered 2-3 fold.

- Urine, Serum, Plasma and Culture Medium Samples: Urine, serum and plasma may be tested directly or diluted with Lysis Buffer. Culture medium can also be tested with dilutions in Lysis Buffer. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.
- Cell Samples: Aspirate medium. Add 1 ml of Lysis Buffer for every 35 cm^2 of surface area. Incubate at 4ºC for 20 minutes. Scrape cells off the surface with a cell scraper. Dissociate sample by pipetting up and down until suspension is homogeneous. Transfer to a centrifuge tube and centrifuge at top speed for 10 min. The supernatant can be assayed directly. Protein concentration >1 mg/ml is recommended for reproducible results. To acetylate the sample, add 10 µL of

Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.

- Tissue Samples: Cyclic nucleotides may be metabolized quickly in tissue, so it is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen). Weigh the frozen tissue and add 5-10 µL of Lysis Buffer per mg of tissue. Homogenize the sample on ice using a Polytron-type homogenizer. Spin at top speed for 5 min and collect the supernatant. The supernatant may be assayed directly. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each cGMP sample, cGMP Standard, and blank should be assayed in duplicate.

   Note: cGMP samples must be compared with corresponding standards (i.e. acetylated samples compared with acetylated standards; non-acetylated samples with non-acetylated standards).
3. Add 50 µL of cGMP sample or standard (acetylated or non-acetylated) to the Goat Anti-Rabbit Antibody Coated Plate.
4. Add 25 µL of diluted Peroxidase cGMP Tracer Conjugate (see Preparation of Reagents Section) to each tested well.
5. Add 50 µL of diluted Rabbit Anti-cGMP Polyclonal Antibody (see Preparation of Reagents Section) to each tested well.
6. Cover with a Plate Cover and incubate at room temperature for 2 hours with shaking.
7. Remove Plate Cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature for 5-20 minutes on an orbital shaker.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical cGMP ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

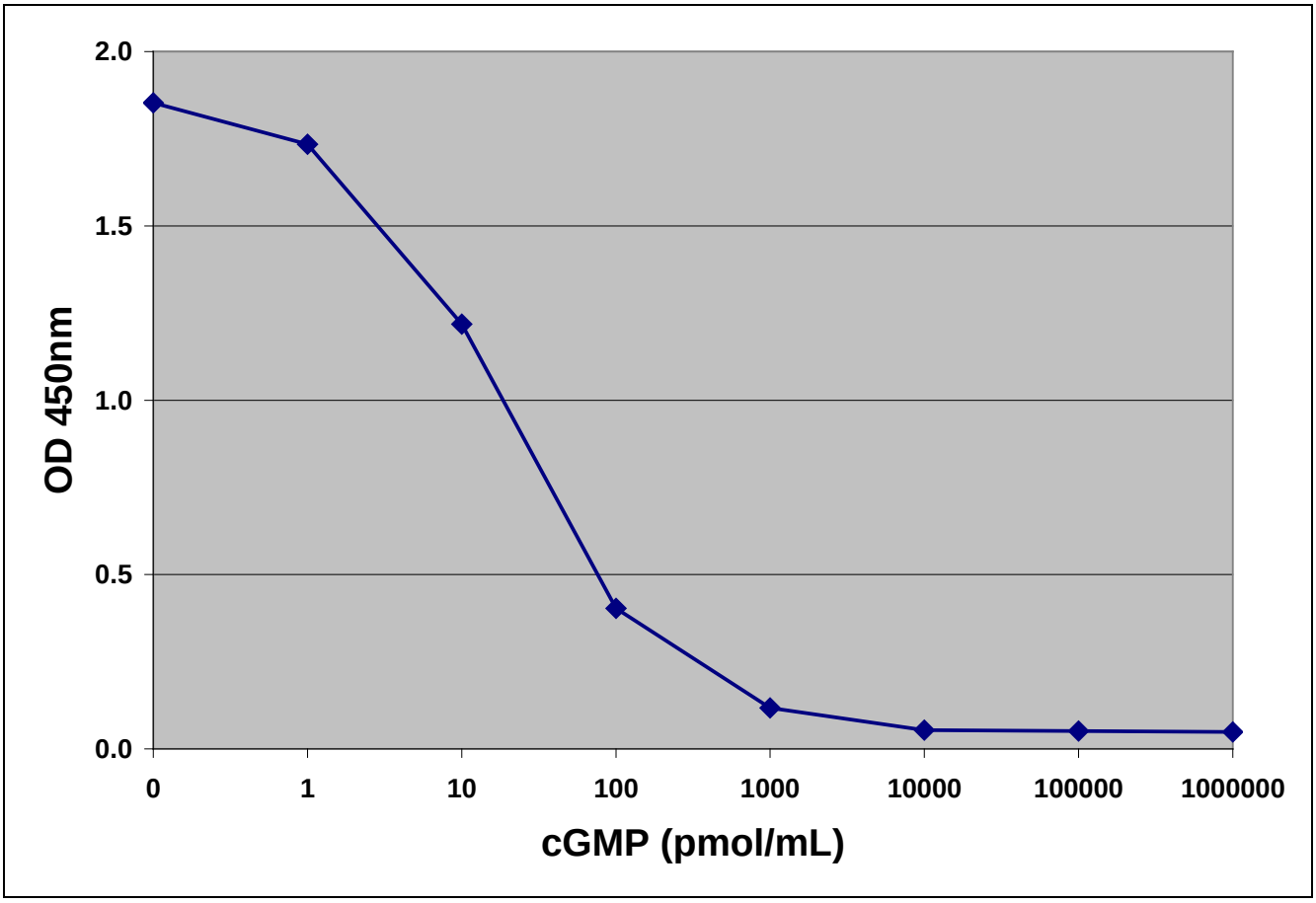


Figure 1: cGMP ELISA Standard Curve (Non-Acetylated Version)

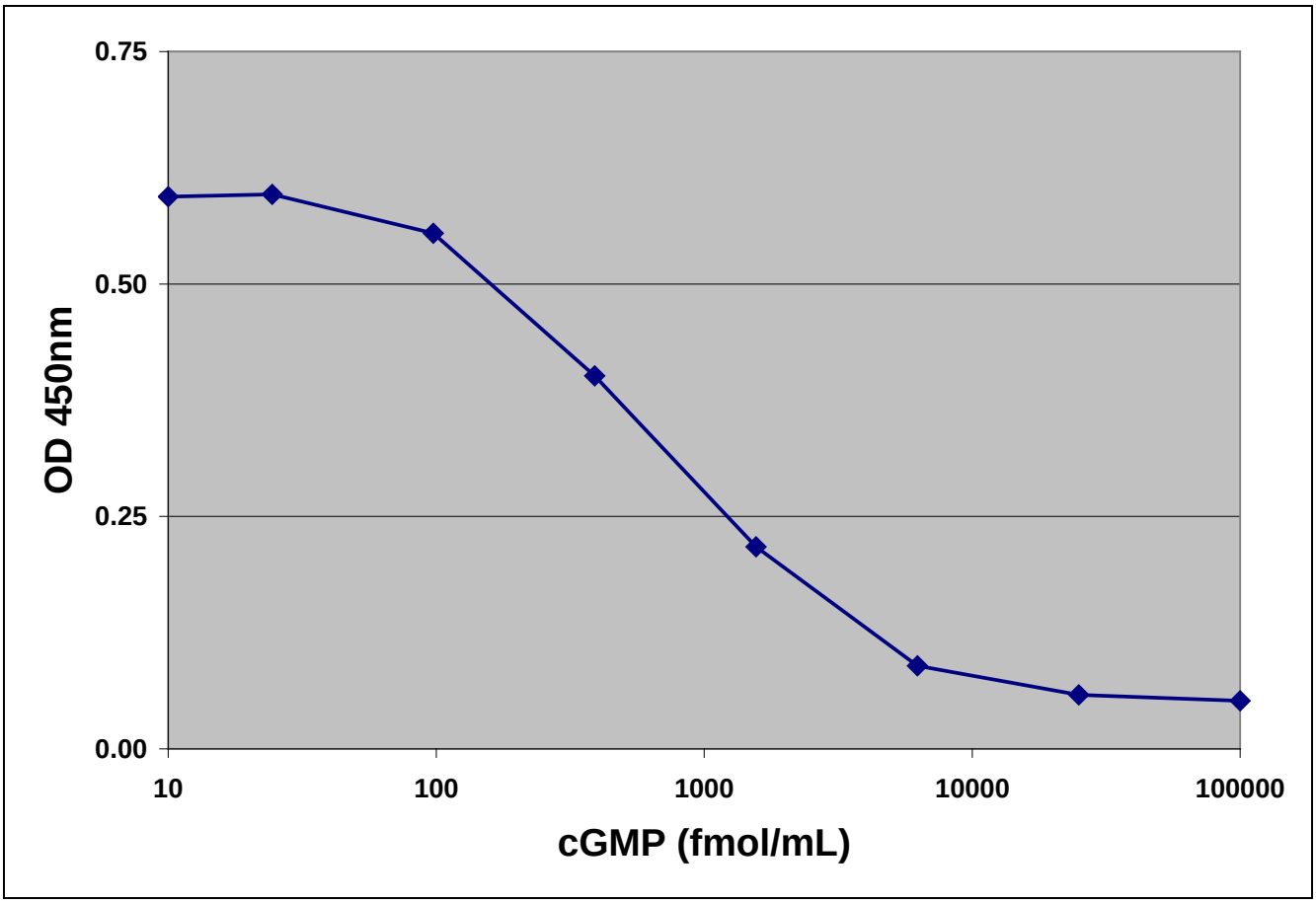


Figure 2: cGMP ELISA Standard Curve (Acetylated Version)

Cross reactivity of cGMP ELISA Kit

Compounds	Cross Reactivity
cGMP	100%
cAMP	<0.1%
AMP	<0.01%
ADP	<0.01%
ATP	<0.01%
GMP	<0.01%
GTP	<0.01%
CTP	<0.01%

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Recent Product Citations

1. Santhanam, A. V. et al. (2014). Erythropoietin increases bioavailability of tetrahydrobiopterin and protects cerebral microvasculature against oxidative stress induced by eNOS uncoupling. *J Neurochem.* **131**:521-529.
2. d'Uscio, L.V. et al. (2014). Mechanisms of vascular dysfunction in mice with endothelium-specific deletion of the PPAR-delta gene. *Am. J. Physiol. Heart Circ. Physiol.* **306**:H1001-H1010.

Warranty

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