APExBIO
An Apoptosis and Epigenetics Company

Product Information

cGMP Direct Immunoassay Kit (Colorimetric)

I. Kit Contents:

Components	K2066-100 100 assays	Cap Color	Storage Temperature	Part Number
10X cGMP Assay Buffer	25 ml	WM	+4℃	K2066-C-1
Standard cGMP (10 nmol)	1 vial	Yellow	-20℃	K2066-C-2
Neutralizing Buffer	7.5 ml	NM	+4℃	K2066-C-3
Acetylating Reagent A	0.75 ml	Violet	+4℃	K2066-C-4
Acetylating Reagent B	1.5 ml	Black	+4℃	K2066-C-5
Anti-cGMP pAb/BSA	1 vial	Red	-20℃	K2066-C-6
cGMP-HRP/BSA	1 vial	Green	-20℃	K2066-C-7
HRP Developer	10 ml	Amber	+4℃	K2066-C-8
Protein G Coated Plate	1 each	---	-20℃	K2066-C-9

II. Introduction:

Cyclic guanosine monophosphate (cGMP) is a second messenger that plays an important role in intracellular signal transduction through the activation of protein kinases and is synthesized from GTP by guanylate cyclase.

The cGMP Direct Immunoassay Kit (Colorimetric) provides a highly sensitive and convenient way for detection of cGMP level in various biological samples based on direct competitive immunoassay and colorimetric method. The recombinant Protein G coated 96-well plate is utilized to efficiently anchor cGMP polyclonal antibody on the plate. Then, cGMP-HRP competes with cGMP from samples for binding to the cGMP polyclonal antibody on the plate. After incubation and washing, the amount of cGMP-HRP bound to the plate can be easily determined by reading HRP activity at OD450 nm. The intensity of absorbance is inversely proportional to the concentration of cGMP in samples. In addition, the assay provides a new acetylation method that significantly improves detection sensitivity. The assay can detect 0.04 -10 pmol/well (0.008 - 2 μM) cGMP levels.

III. Reagent Preparation:

Dilute the 10X cGMP Assay Buffer to 1X Assay Buffer with MilliQ water. Store at 4℃.

Reconstitute the Standard cGMP (pellet may not be visible) in 1 ml of 0.1M HCl (not provided), vertex for 10 seconds to generate 10 pmol/µl cGMP stock standard solution.

Reconstitute rabbit anti-cGMP pAb and cGMP-HRP each with 1.1 ml of the 1X Assay Buffer as stock solutions.

Unused well strips can be kept at –20℃ with the desiccants, stable for up to 1 month.

The kit should be stored at –20℃. After reconstitution, some components may be stored at 4℃ as instructed above, stable for up to 1- 2 months.

NOTE- Acetylating Reagent B is very volatile and hence the vial has to be tightly capped and stored only at +4℃.

IV. General Consideration:

cGMP samples in 0.1 M HCl (final concentration) is stable and can be used directly in the assay. Make dilutions of your sample with 0.1 M HCl to the range of 0.04 - 10 pmol/well (0.008 - 2 µM).

Plasma, serum, whole blood, and tissue homogenates often contain phosphodiesterases and large amount of immunoglobulins (IgGs) which may interfere with the assay. However, diluting these samples with 0.1M HCl can generally inactivate phosphodiesterases and lower the concentration of IgGs, making the samples suitable for the assay. Phosphodiesterases and IgGs can also be removed by 5% TCA precipitation or 10 kD molecular weight cut off micro centrifuge filters.

To determine whether interference is presence in your sample, you may make two different dilutions. If the two different dilutions of sample show good correlation in the final calculated cGMP concentrations, purification is not required; otherwise use TCA precipitation or 10 Kd molecular weight cut off microcentrifuge filters.

Some organic solvents may interfere with the assay and may need to be removed prior to the assay.

V. Sample Preparation:

Urine, Plasma and Culture Media: Urine, plasma, and culture media may be tested directly after adding 1/10 volume of 1M HCl, and remove precipitates if occur.

Culture Cells: For suspension cells collect by centrifugation. Add 1 ml of 0.1M HCl for every 35 cm^2 of surface area (e.g., 10 cm plate at 70 % confluency is ~ 110 cm^2, so use ~ 3.1 ml). Incubate at room temperature for 20 minutes on ice. For adherent cells add the HCL directly, scrape cells off the surface. 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 volume of 0.1M HCl. 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.

VI. cGMP Assay Protocol:

Prepare cGMP Standard Curve and Samples:

1. Add 200 µl of the 10 pmol/µl standard cGMP stock into 800 µl of 0.1M HCl to generate 2 pmol/µl cGMP working solution. The diluted cGMP should be used within 1 hour.
2. Label 11 microcentrifuge tubes, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039, 0, 0_B pmol/50 µl. (Note: these concentrations represent what will finally be in the wells after the dilutions mentioned below).
3. Add 200 µl of the 2 pmol/µl cGMP into the tube labeled 10 pmol (enough for 20 tests), add100 µl 0.1M HCl into the rest of tubes.
4. Transfer 100 µl from the 10 pmol tube into the labeled 5 pmol tube, mix. Continue the serial dilution by transferring 100 µl from the 5 pmol tube to 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039 pmol tubes. Discard 100 µl from the 0.039 pmol tube. The diluted cGMP should be used within 1 hour.
5. Label new tubes for test samples, add 100 µl each test sample per tube. We suggest using different dilutions for each sample (dilute with 0.1M HCl).
6. Add 50 µl of Neutralizing Buffer to each tube to neutralize the HCl in the samples and standards.
7. Prepare Acetylating Reagent Mix (Note: 5 µl is needed for each assay): Mix 1 volume of Acetylating Reagent A (Violet cap) with two volumes of Acetylating Reagent B (Black cap) in a microtube. Prepare just enough for the experiment. Use within 1 hour.
8. Add 5 µl of the Acetylating Reagent Mix directly into each test solution (both standard and sample), IMMEDIATELY vortex 2-3 seconds following each addition without delay, one tube at a time and incubate at room temperature for 10 min.
9. Add 845 µl 1X Assay Buffer into each tube, mix well. Use for below quantification.

Note: The acetylation step improves the assy sensitivity significantly and avoid the interferences of many components in unpurified samples. (If cGMP in your samples are very low, the acetylation reagents can be dried after step 8, without dilution step 9 to minimize the volume. Then reconstituted in a 50 - 100 µl volume of Assay Buffer).

Quantification cGMP:

1. Add 50 µl of the acetylated Standard cGMP and test samples from Step 9 above to each well of the Protein G coated 96-well plate. We suggest duplicate assays for each sample and standard.
2. Add 10 µl of the reconstituted cGMP antibody per well to the standard cGMP and sample wells except the well with 0_B pmol cGMP. (Note: Do not add cGMP antibody into the well with 0_B pmol cGMP, instead add 10 µl of 1X Assay Buffer for background reading). Incubate for 1 hour at room temperature with gentle agitation.

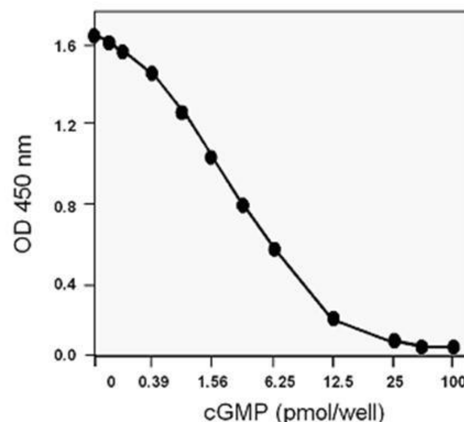
Note: Using a repeating pipette is recommended for minimizing pippetting errors.

3. Add 10 µl of cGMP-HRP to each well and incubate for 1 hr at room temperature with gentle agitation.
4. Wash 5 times with 200 µl 1X Assay Buffer each time. Completely empty the wells by tapping the plate on a fresh paper towel after each wash step.
5. Add 100 µl of HRP developer and develop for 1 hour at room temperature with agitation.
6. Stop the reaction by adding 100 µl of 1M HCl (not provided) to each well (sample color should change from blue to yellow).
7. Read sample at $OD_{450\ nm}$.
8. Subtract $OD_{450\ nm}$ background reading (the well with 0_B pmol cGMP) from all samples and standards. Plot standard curve to observe the linear portion, then replot only the linear portion and in Excel add a trendline, then use the trend line linear formula (y=mx+b). Calculate amount of cGMP in samples after correcting the for dilution factors.
9. Calculations:

$$C = Sa/Sv \text{ pmol/µl or nmol/ml or µM.}$$

Where: Sa is the cGMP amount (pmol) from the Standard Curve.

Sv is the sample volume (µl) added into the assay wells after dilution factor correction.



cGMP Standard Curve: The assay was performed following the kit protocol

Frequently Asked Questions

1. Guanosine 3,5-cyclic monophosphate (cyclic GMP, cGMP) has been shown to be present at levels typically 10 - 100 fold lower than cAMP in most tissues. How does this fit with the cAMP Direct Immunoassay Kit to be much more sensitive, 1 ~ 100 fmol of cAMP per assay, than the cGMP Direct Immunoassay Kit, 0.1 - 10 pmol cGMP per assay?

The cAMP antibody affinity is higher than that of cGMP. That is the reason why you need fmol of cAMP while for cGMP you need pmol quantities.

2. Would the release of hemoglobin from lysed RBC interfere with the read-out?
The hemoglobin will not bind to the antibody in the plate. It will go out with the wash.

3. How stable is cGMP in 0.1 M HCl?
cGMP is very stable in 0.1 M HCl.

4. Can I freeze my cell lysate at -70℃ and test the cGMP levels later?
Yes

5. How much sample do we need?
10 Million cells or 100 mg of tissue

6. How does the plotting of the standard curve works?
The Abs is reverse proportionally to cGMP dose, so Abs of 10 pmol/ul is lower than the 0 pmol/ul. Acetylation step increase signal very significantly. Stop the reaction by adding 100ul 1M HCl may also double the signal.

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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