Direct Competitive cGMP ELISA Kit
Based on Monoclonal Anti-cGMP Antibody

Introduction

Guanosine 3',5'-cyclic monophosphate (cGMP) is a key second messenger molecule in intracellular signal transduction. Monitoring cGMP levels is one of the most common ways to screen for agonists and antagonists of G protein–coupled receptors(GPCRs). Bioyeargene cGMP ELISA Kit is based on the competition between HRP-labeled cGMP and free cGMP for fixed amounts of mouse monoclonal anti-cAMP antibody(cGMP MAb) binding sites. Because the concentration of the HRP-labeled cGMP is held constant while the concentration of cGMP varies, the amount of HRP-labeled cGMP that is able to bind to the cGMP MAb will be inversely proportional to the concentration of cGMP in the well. This antigen-antibody complex binds to the goat anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then developed. The ELISA typically displays an IC50 (50% B/B0) of approximately 5 pmol/mL and a detection limit of approximately 0.1 pmol/mL.

Reagents & Materials Provided

Component	Product #	Storage
1. cGMP Standard 10000 pmol/mL cGMP, 1×0.1mL	Bk021	-80°C
2. cGMP-HRP 1000× stock of HRP conjugated with cAMP, 1×0.02mL	Bk022	-80°C
3. Anti-cGMP McAb Anti-cAMP monoclonal antibody (1000× stock), 1×0.02mL	Bk023	-80°C
4. 10×Assay Buffer phosphate buffer with preservative, 1×30mL	Bk004	2-8°C
5. Neutralizing Reagent tris buffered saline with preservative, 1×8mL	Bk005	2-8°C
6. Sample Diluent 0.1M HCl, only required for extraction of samples and diluting standard, 1x30mL	Bk006	2-8°C
7. TMB Substrate 3,3',5,5'Tetramethylbenzidine, 1×18mL	Bk007	2-8°C
8. Stop Solution 2M sulphuric acid, 1×8mL	Bk008	2-8°C
9. Goat anti-Mouse IgG Coated plate of 96 wells 12×8 well strips in a bag with desiccant	Bk009	2-8°C

Storage & Stability

Store kit at 4°C immediately upon receipt, apart from Standard、HRP conjugate、antibody, which should be stored at -80°C.

MATERIALS REQUIRED, NOT SUPPLIED

Standard microplate reader - capable of reading at 450 nm, preferably with correction at 630 nm

Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed

Eppendorf tubes

Microplate Shaker

Absorbent paper for blotting

Deionized water

Sample handling

Plasma, serum, whole blood, tissue homogenates and other samples often contain phosphodiesterases and large amount of proteins (e.g.globulins, albumin)which may interfere with the assay. However, preparing samples in 0.1M HCl can generally inactivate phosphodiesterases and lower the concentration of proteins, making the samples suitable for the assay. Both phosphodiesterases and proteins can also be removed by 5% TCA precipitation or by using 10 kDa molecular weight cut off microcentrifuge filters.

Tissue samples: It is important to rapidly freeze tissues using liquid nitrogen after collection of tissue samples. Then weigh the frozen tissue and add 5-10 volume of 0.1M HCl. Homogenize the sample on ice using a homogenizer. Centrifuge at 3000 rpm at room temperature for 5min. The supernatant may be assayed directly and can then be diluted in the 0.1M HCl.

Cells samples: After removing the culture medium, cells can be collected and treated with 0.1M HCl. Incubate at room temperature for 20 minutes and ensure complete cell lysis. Centrifuge at 3000 rpm at room temperature for 5min, then the supernatants may be used directly in the assay.

Urine, Serum and Culture Medium Samples: After treating 1mL of the supernatant media with 10 μL of concentrated hydrochloric acid, Centrifuge Urine and plasma for 5 minutes at 3000 rpm at room temperature. The supernatants can then be used directly in the assay or diluted to a suitable multiple.

Procedural notes

1. Bring all kit reagents to warm to room temperature(26±2°C) before use.
2. Pipet standards and samples to the bottom of the wells.

3. Add the reagents to the side of the well to avoid contamination.

4. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.

5. Prior to addition of substrate, ensure that there is no residual wash buffer in the wells.

6. Stop solution is caustic; care should be taken in use.

Reagent preparation

1. cGMP standard solution

   Label eight tubes 1# through 8#. Pipet 990 μL 0.1M HCl into tube 1# and 500 μL 0.1M HCl into tubes 2-8#. Add 10 μL of the 10000 pmol/mL standard to tube 1#. Vortex thoroughly. Add 500 μL of tube 1# to tube 2# and vortex thoroughly. Continue this for tubes 3# through 8#.
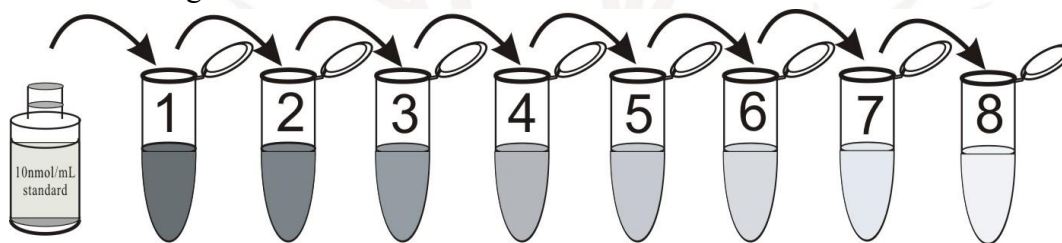
   The concentration of cGMP in tube 1# through 9# will be 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 pmol/mL respectively. Diluted standards should be used within 15 minutes of preparation. Label one tube as the Zero Standard(0 pmol/mL, maximum binding/B_0). Pipet 500 μL 0.1M HCl into this tube.

2. Assay buffer

   prepare the wash buffer by diluting 30 mL of the supplied concentrate with 270 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

3. cGMP-HRP conjugate

   Make 1:1000 dilution with assay buffer to have 1× HRP-cGMP conjugate working solution before use.

4. Conjugate 1:20 Dilution for Total Activity Measurement

   Prepare the Conjugate 1:20 Dilution by diluting 10 μL of the Conjugate working solution with 190 μL of the assay buffer. This 1:20 dilution is intended for use in the Total Activity wells only.

5. Anti-cGMP McAb

   Make 1:1000 dilution with assay buffer to have 1× Anti-cGMP McAb conjugate working solution before use.

Assay procedure

Add various reagents to the wells according to the following table:

Well	Neutralizing Buffer	0.1M HCl	Standard /Sample	cGMP - HRP Conjugate	Anti-cGMP MAb	Assay Buffer
Blank	-	-	-	-	-	-
TA	-	-	-	5 µL	-	-
NSB	50 µL	50 µL	-	50 µL	-	50 µL
B_0	50 µL	50 µL	-	50 µL	50 µL	-
Standard /Sample	50 µL	-	50 µL	50 µL	50 µL	-

1. Add 50 µL of the Neutralizing Buffer to each well, except the TA (Total Activity) and Blank wells.

2. Pipet 50 µL of 0.1M HCl into the NSB (Non-Specific Binding) and the maximum binding (B_0) wells.

3. Pipet 50 µL of Standards and samples to the appropriate wells.

4. Pipet 50 µL of assay buffer into the NSB wells.

5. Pipet 50 µL of Conjugate into each well except the TA and Blank wells.

6. Pipet 50 µL of Antibody into each well, except the Blank, TA and NSB wells.

7. Incubate the plate at room temperature (26±2℃) for 2 hours.

8. Wash 4 times with 260 µL Assay Buffer each well. Completely empty the wells by tapping the plate on a new paper towel.

9. Add 5 µL of the Conjugate to the TA wells.

10. Add 150 µL of the Substrate solution to each well. Incubate at room temperature for about 10 minutes without shaking.

11. Stop the reaction by adding 50 µL of Stop Solution to each well and read at 450 nm.

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	S1	S1	①	⑯	⑰	㉜	㉝	㊽	㊾	㉛	㉟
B	blank	S2	S2	②	⑮	⑱	㉛	㉞	㊼	㊿	㉓	㊻
C	NSB	S3	S3	③	⑭	⑲	㉚	㉟	㊻	�51	㉒	㊿
D	NSB	S4	S4	④	⑬	⑳	㉙	㊱	㊺	㊼	㉑	㊽
E	B_0	S5	S5	⑤	⑫	㉑	㉘	㊲	㊹	㊽	⑳	㊾
F	B_0	S6	S6	⑥	⑪	㉒	㉗	㊳	㊸	㊾	㊾	㊀
G	B_0	S7	S7	⑦	⑩	㉓	㉖	㊴	㊷	㊾	㊽	㊁
H	TA	S8	S8	⑧	⑨	㉔	㉕	㊵	㊶	㊾	㊼	㊂

S1-S8-Standards 1-8   ①-㊂-Sample

Calculation & Standard Curve

Calculate the average Net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

Average Net OD = Average Bound OD $-$ Average NSB OD

The data should be plotted as B/B_0 versus log concentration using curve fitting routines such as exponential logistic curve fitting or three order polynomial linear regression curve-fit.

Calculate the B/B_0 value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot.

The lower limit of detection was determined as the concentration corresponding to a signal two standard deviations above the mean of the zero standard. The LOD is ~0.1 pmol/mL.