Direct Competitive cGMP ELISA Kit
Catalogue Number: OM700063
(96Tests)
Store all reagents at 2-8°C
Collect sample: serum or blood plasma
Assay range ： 0.78 -100 pmol/mL

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

This Exenatide ELISA kit is intended for laboratory research use only and is not to be used for diagnostic or therapeutic procedures. The Stop Solution changes the color of the solution from blue to yellow, after which the color intensity is measured spectrophotometrically at a wavelength of 450 nm. To determine the the concentration of Exenatide in the samples, this Exenatide ELISA Kit is provided with a set of calibration standards. The calibration standards are assayed at the same time as the samples, allowing the operator to plot a standard curve of optical density versus the corresponding Exenatide concentration. By comparing the optical density of the samples to this standard curve, the concentration of Exenatide in the samples can be determined.

PRINCIPLE OF THE ASSAY

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 PROVIDED

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

1. Goat anti-Mouse IgG Coated plate	96 wells	1 plate
2. Anti-cGMP McAb（1000×stock ）	0.02 mL	1 tube
3. cGMP Standard (10000 pmol/mL)	0.1 mL	1 tube
4.cGMP-HRP（1000 x stock）	0.02 mL	1 tube
5. 10x Assay Buffer	30 mL	1 tube
6. Neutralizing Reagent	8 mL	1 vial
7. Sample Diluent	30 mL	1 vial
8. TMB Substrate	18 mL	1 vial
9. Stop Solution	8 mL	1 vial
10. Instruction	1 copy	

SAMPLE COLLECTION AND STORAGE

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 3000rpm 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 5 min, 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.

DO NOT USE HEAT-TREATED SPECIMENS.

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.

PRECAUTIONS

1. Do not interchange or mix reagents from different kit lots. The standards, labels, and microplate are matched to ensure optimal performance; use only the reagents provided by the manufacturer.
2. Bring all reagents and materials to room temperature (20-25°C) before use. Do not thaw samples or reagents using a water bath.
3. Do not use any kit components beyond their expiration date.
4. Use only deionized or distilled water for diluting reagents.
5. Remove the microplate strips from the protective pouch only immediately before use. Any unused strips should be resealed in the original pouch with the desiccant and stored at 2-8°C.
6. Use a fresh, disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid with sodium hypochlorite solutions.
8. Serum and plasma should be regarded as potentially hazardous materials capable of transmitting infectious agents. Disposable gloves must be worn during the assay procedure, as no method can fully guarantee that canine blood-derived products are free of transmissible pathogens. Therefore, all blood-derived products should be handled as potential sources of infection in accordance with good laboratory practices.
9. All samples should be processed using methods capable of inactivating viruses.
10. Solid Waste: Autoclave at 121°C for 60 minutes.
11. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste liquid must stand for at least 30 minutes to inactivate viruses before disposal.
12. Substrate Solution is susceptible to contamination. Do not use if it appears

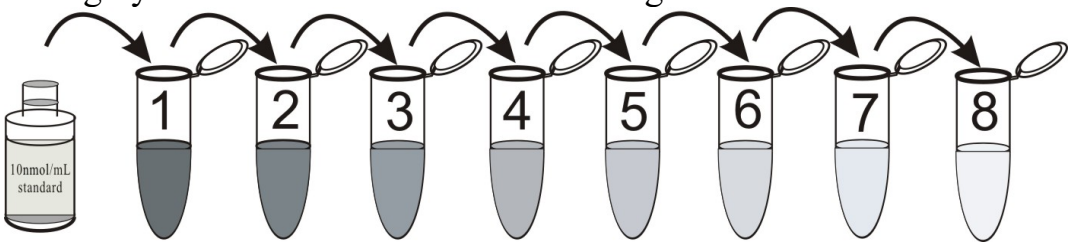
blue before use.

13. Chromogen Solution B contains 20% acetone. Keep away from heat sources or open flames.
14. Remove all kit reagents from the refrigerator and allow them to equilibrate to room temperature (20-25°C).

REAGENT PREPARATION

1. cGMP standard solution

Label eight tubes 1# through 8#. Pipet 990 µ L 0.1M HCl into tube 1# and 50 µ L 0.1M HCl into tubes 2-8#. Add 10 µ L of the 10000 pmol/mL standard to tube 1#. Vortex thoroughly. Add 500 µ L 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/B0). Pipet 500 µ L 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× cGMP-HRP 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
B0	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(B0) 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	㊹	㊺
B	Blank	S2	S2	②	⑮	⑱	㉛	㉞	㊼	㊿	㊸	㊻
C	NSB	S3	S3	③	⑭	⑲	㉚	㉟	㊻	㊶	㊷	㊼
D	NSB	S4	S4	④	⑬	⑳	㉙	㊱	㊺	㊷	㊶	㊽
E	B_0	S5	S5	⑤	⑫	㉑	㉘	㊲	㊹	㊸	㊿	㊾
F	B_0	S6	S6	⑥	⑪	㉒	㉗	㊳	㊸	㊹	㊾	㊿
G	B_0	S7	S7	⑦	⑩	㉓	㉖	㊴	㊷	㊺	㊽	㊶
H	TA	S8	S8	⑧	⑨	㉔	㉕	㊵	㊶	㊻	㊼	㊷

S1-S8-Standards 1-8 ①-㉒-Sample

TYPICAL DATA

CALCULATION OF RESULTS

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.

