ab285321 – cGMP ELISA Kit

For in vitro quantitative determination of cGMP concentrations in serum, plasma and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285321

Storage and Stability

An unopened kit can be stored at 4°C for 12 months. Once the kit is opened, please store the items separately according to the recommended conditions below.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 wells ×12 strips	-20°C
Standard	2 vials	-20°C
100X Biotinylated Detection Antibody	120 µl	-20°C
100X HRP Conjugate	120 µl	-20°C
Standard & Sample Diluent	20 mL	4°C
Biotinylated Detection Ab Diluent	14 mL	4°C
HRP Conjugate Diluent	14 mL	4°C
25X Wash Buffer	30 mL	4°C
Substrate Reagent	10 mL	4°C
Stop Solution	10 mL	4°C
Plate Sealer	5 units	N/A

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader with 450 nm wavelength filter
- Deionized or distilled water

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.
Δ Note: if crystals have formed, incubate it in a 40°C water bath and mix it gently until the crystals have completely dissolved.

100X Biotinylated Detection Antibody: Calculate the required amount before the experiment (50µL/well). Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent. Bring all reagents to room temperature (18~25°C) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.

100X HRP Conjugate: Calculate the required amount before the experiment (100 µL/well). Dilute the 100×Concentrated HRP Conjugate to 1×working solution with Concentrated HRP Conjugate Diluent.

Standard Preparation

1. Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times.
2. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 50 pmol/mL.
3. Prepare serial dilutions as needed. Suggested standard points are: 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0 pmol/mL.
4. Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube.
5. Pipette 500 µl of the 50 pmol/mL stock solution to the first tube and mix up to produce a 50 pmol/mL working solution.
6. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.

Sample Preparation

- Samples should be assayed within 7 days when stored at 4°C, otherwise aliquot and stored at -2°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and endotoxin free.

Plasma: Collect plasma using EDTA-Na2 as anticoagulant. Centrifuge samples for 15 min at 1000× g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Haemolysed samples are not suitable for ELISA assay.

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 µL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10min at 1500×g at 4°C. Remove the cell fragments, collect the supernatant for assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from the literature before analysing different tissue types. For general information, haemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000×g to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g at 2~8°C. Collect the supernatant for assay.

Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve must be run with each assay.

1. Add 50 µL of each standard or samples into appropriate wells.
2. Immediately add 50 µL of Biotinylated Detection Antibody working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
3. Aspirate the solution from each well add 350 µl of 1x wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.

4. Add 100 µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 2.
6. Add 90 µL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a microplate reader set to 450 nm.

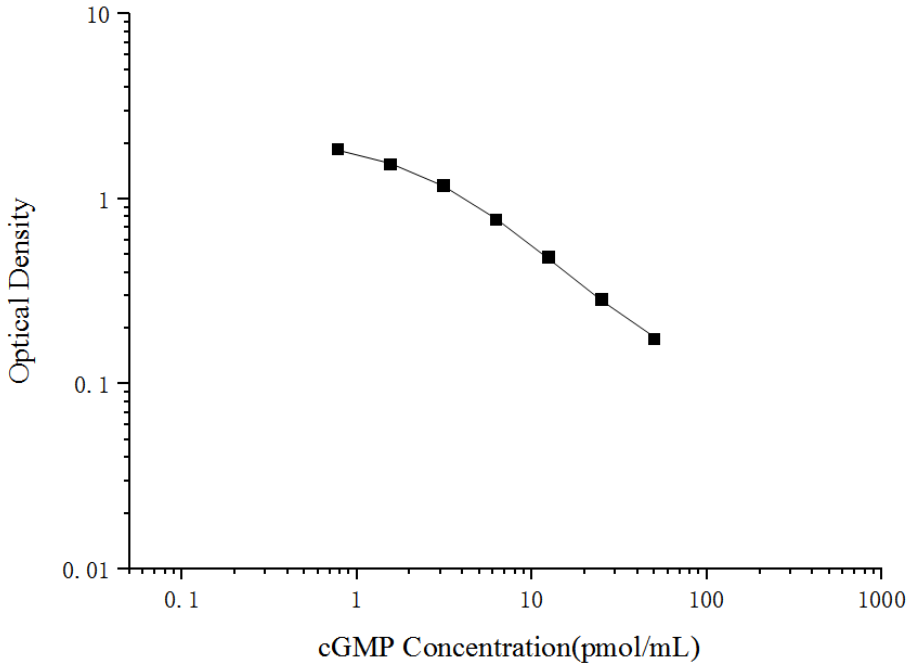
Calculation

- Determine the average of the duplicate readings for each standard and samples.
- Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis.
- If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.
- If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution.
- The actual concentration is the concentration obtained by calculation multiplied by the dilution factor.

Typical data

- As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only:

pmol/mL	OD
50	0.174
25	0.285
12.5	0.483
6.25	0.774
3.13	1.183
1.56	1.538
0.78	1.853
0	2.162



Technical Support

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Version 3a, Last updated October 28, 2025