ab133052 – Cyclic GMP Complete ELISA Kit

Instructions for Use

For quantitative detection of Cyclic GMP Complete in cells and tissue treated with 0.1N HCl, in addition to culture supernatants, saliva, and serum.

This product is for research use only and is not intended for diagnostic use.

Version 5a Last Updated 19 May 2022

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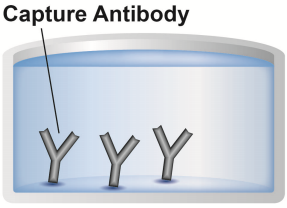
1. BACKGROUND

Abcam's Cyclic GMP Complete *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Cyclic GMP Complete in cells and tissue treated with 0.1N HCl, in addition to culture supernatants, saliva, and serum.

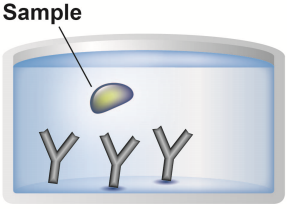
A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-cGMP antigen and a polyclonal rabbit antibody specific to cGMP. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of cGMP captured in the plate.

Guanosine 3',5'-cyclic monophosphate (cyclic GMP) was identified in 1963. It has been shown to be present at levels typically 10-100 fold lower than cAMP in most tissues and is formed by the action of the enzyme guanylate cyclase on GTP. It is involved in a number of important biological reactions. Some hormones, such as acetylcholine, insulin, and oxytocin, as well as certain other chemicals like serotonin and histamine cause an increase in Cyclic GMP levels. Stimulators of guanylate cyclase such as the vasodilators niroprusside, nitroglycerin, sodium nitrate, and nitric oxide (NO) also stimulate cGMP levels. Peptides, such as atrial natriuretic peptide (ANP) that relax smooth muscle also increase Cyclic GMP concentrations. Cyclic GMP has been confirmed as a second messenger for ANP6. NO can be synthesized from L-arginine and diffuse through cell membranes. The interaction of NO with guanylate cyclase allows cGMP to act as a third messenger in some cells.

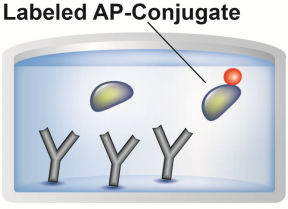
2. ASSAY SUMMARY



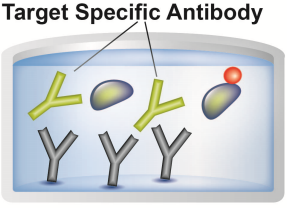
Prepare all reagents and samples as instructed.



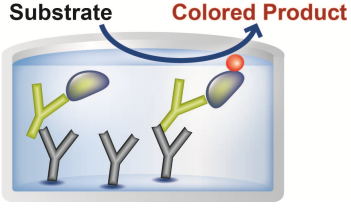
Add standards and samples to appropriate wells.



Add prepared labeled AP-conjugate to appropriate wells.



Add Cyclic GMP Complete antibody to appropriate wells. Incubate at room temperature.



Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance
- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the Alkaline Phosphatase Conjugate and Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 Wells	2-8ºC
Cyclic GMP Complete Alkaline Phosphatase Conjugate	5 mL	-20ºC
Cyclic GMP Complete Antibody	5 mL	2-8ºC
Cyclic GMP Complete Standard	500 µL	-20ºC
20X Wash Buffer Concentrate	27 mL	2-8ºC
pNpp Substrate	20 mL	2-8ºC
Stop Solution	5 mL	2-8ºC
Acetylation kit - Triethylamine	2 mL	2-8ºC
Acetylation kit - Acetic Anhydride	1 mL	2-8ºC
Assay Buffer 2	27 mL	2-8ºC
0.1M HCl	27 mL	2-8ºC
Neutralizing Reagent	5 mL	2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- Triton X-100 (optional for sample preparation)
- Optional (for tissue samples): Liquid nitrogen, mortar & pestle, and concentrated HCl

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- 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
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 **Cyclic GMP Complete Alkaline Phosphatase Conjugate**

Allow the Cyclic GMP Complete Alkaline Phosphatase Conjugate to warm to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 **1X Wash Buffer**

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

9.3 **Acetylation Reagent (optional)**

Prepare the Acetylating Reagent by adding 0.5 mL of Acetic Anhydride to 1 mL of Triethylamine. Note that this volume is sufficient to add to 30 mL of diluted standards and samples. Use the prepared reagent within 60 minutes of preparation.

Discard any unused portion of the Acetylating Reagent.

10. STANDARD PREPARATIONS – NON-ACETYLATED FORMAT

Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation. Prepare serially diluted standards immediately prior to use.

10.1 For:

10.1.1 **Serum, plasma, urine, or saliva:** dilute the Cyclic GMP standards with Assay Buffer 2.

10.1.2 **Culture Supernatants:** use the same non-conditioned media for the standard diluent.

10.1.3 **Cell lysates** and **Tissue samples** prepared in 0.1M HCL: use the supplied 0.1M HCL as the standard diluent.

10.2 Allow the reconstituted 5,000 pmol/mL Cyclic GMP Stock Standard solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.3 Label six tubes with numbers 1 – 5 and one with B_O.

10.4 Prepare a 500 pmol/mL **Standard 1** by transferring 100 µL of 5,000 pmol/mL Stock Standard to 900 µL of appropriate sample diluent in tube 1. Mix thoroughly and gently.

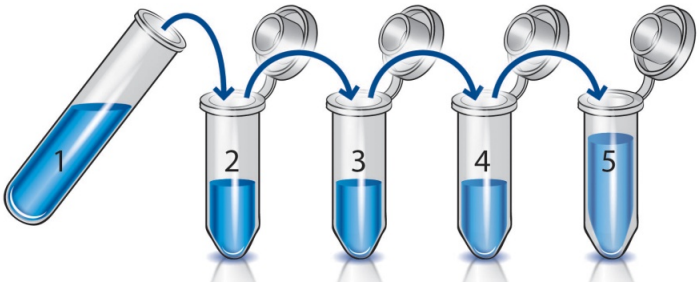
10.5 Add 800 µL of the appropriate sample diluent in tubes numbers 2-5.

10.6 Prepare **Standard 2** by transferring 200 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.7 Prepare **Standard 3** by transferring 200 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.8 Using the table as a guide, repeat for tubes 4 and 5.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pmol/mL)	Final Conc. (pmol/mL)
1	Standard	100	900	5,000	500
2	Standard 1	200	800	500	100
3	Standard 2	200	800	100	20
4	Standard 3	200	800	20	4
5	Standard 4	200	800	4	0.8
B_O	None	-	800	-	-



11. STANDARD PREPARATIONS –ACETYLATED FORMAT (optional)

Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation. Prepare serially diluted standards immediately prior to use.

11 **For:**

11.1.1 **Serum, plasma, urine, or saliva**: dilute the Cyclic GMP standards with Assay Buffer 2.

11.1.2 **Culture supernatant**: use the same non-conditioned media for the standard diluent.

11.1.3 **Cell lysates and Tissue samples** prepared in 0.1M HCL: use the supplied 0.1M HCL as the standard diluent.

11.2 Allow the reconstituted 5,000 pmol/mL Cyclic GMP Stock Standard solution to warm to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

11.3 Label six tubes with numbers 1 – 5 and one with B_O.

11.4 Prepare a 50 pmol/mL Standard 1 by adding 10 µL of 5,000 pmol/mL Stock Standard to 990 µL of the appropriate sample diluent into tube 1. Mix thoroughly and gently.

11.5 Add 800 µL of the appropriate sample diluent into tubes numbers 2 – 5.

11.6 Prepare Standard 2 by transferring 200 µL from Standard 1 to tube 2. Mix thoroughly and gently.

11.7 Prepare Standard 3 by transferring 200 µL from Standard 2 to tube 3. Mix thoroughly and gently.

11.8 Using the table as a guide, repeat for tubes 4 and 5.

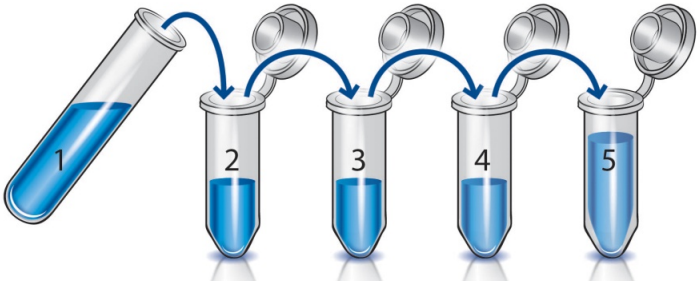
11.9 Acetylate all standards by adding 10 µL of the Acetylating Reagent for each 200 µL of the standard. Add the Acetylating Reagent directly to the diluted standard or sample and vortex immediately after the addition of the Acetylating Reagent.

11.10 Note: If acetylating standards, then samples must be acetylated in the same format by adding 10 µL of the Acetylating Reagent for each 200 µL of the sample.

11.11 Add 1 mL of 0.1M HCl into the B_O tube, followed by 50 µL of the Acetylating Reagent. Use in steps 13.3 and 13.4 of the Assay Procedure.

11.12 If acetylating standards, then samples must be acetylated in the same format by adding 10 µL of the Acetylating Reagent for each 200 µL of the sample.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pmol/mL)	Final Conc. (pmol/mL)
1	Standard	10	990	5,000	50
2	Standard 1	200	800	50	10
3	Standard 2	200	800	10	2
4	Standard 3	200	800	2	0.4
5	Standard 4	200	800	0.4	0.08
B_O	None	-	800	-	-



12. SAMPLE COLLECTION AND STORAGE

- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles
- Samples containing rabbit IgG will interfere with the assay. EDTA plasma may precipitate during acetylation
- Treatment of cells and tissue with the supplied 0.1M HCl will stop endogenous phosphodiesterase activity and allow for the direct measurement of these samples in the assay without evaporation or further processing. Biological fluids, such as serum and saliva, should be diluted in Assay Buffer 2 and run directly in the assay. A minimum 1:10 dilution is required for serum and a 1:4 dilution for saliva. These are the minimum dilutions required to remove matrix interference of these samples
- Please note that some samples may contain high levels of Cyclic GMP Complete and additional dilution may be required. Samples with low levels of Cyclic GMP Complete may be assayed in the acetylated format or the samples may be concentrated

12.1 Protocol for Cell Lysates

The concentration of cells used must be optimized for the specific cell line and treatment conditions. Cells may be grown in typical containers such as Petri dishes, culture plates (e.g., 48-well, 12-well, or 96-well), culture flasks, etc. Some cells are particularly hardy (e.g., bacteria) and may require the addition of 0.1 to 1% Triton X-100 to the 0.1M HCl for enhanced lysis. If Triton X-100 is added to samples it should also be added to the standard dilution as a modest increase in optical density may occur.

12.1.1. Pellet suspension cells and aspirate the media. Treat cells with 0.1M HCl. A general starting concentration of 1 x 10^6 cells per mL of 0.1M HCl is recommended. Remove the media from adherent cells and add enough 0.1M HCl to cover the bottom of the plate. Avoid over-diluting the sample with an excessive

volume of HCl. Please note that the culture media may be saved and assayed separately, if desired.

12.1.2. Incubate the cells in 0.1M HCl for 10 minutes at room temperature.

12.1.3. Inspect the cells under a microscope to ensure uniform lysis. Continue incubating for an additional 10 minutes, if necessary.

12.1.4. Centrifuge ≥600 x g to pellet the cellular debris.

12.1.5. The supernatant may be assayed immediately or stored frozen for later analysis.

Note: Standards must be diluted in 0.1 M HCl and Neutralizing Reagent used.

12.2 Protocol for Tissue Samples

Two options are available for tissue samples.
Protocol 1 is more straightforward and user-friendly. Protocol 2 is available if samples require concentration.

Protocol 1 : Treatment with 0.1 M HCl

1. After collection, tissue samples should be flash frozen in liquid nitrogen. If analysis cannot be carried out immediately, store tissue at -80°C.
2. Grind frozen tissue to a fine powder under liquid nitrogen in a stainless steel mortar.
3. When liquid nitrogen has evaporated, weigh the frozen tissue and homogenize in 10 volumes of 0.1M HCl (e.g., 0.1 g of tissue should be homogenized in 1 mL of 0.1M HCl).
4. Centrifuge ≥600 x g to pellet the debris (~10 minutes).
5. The supernatant may be further diluted in the 0.1M HCl provided and run directly in the assay or stored frozen for later analysis.

Note: Standards must be diluted in 0.1 M HCl and Neutralizing Reagent used.

Protocol 2 : TCA / Ether Extraction

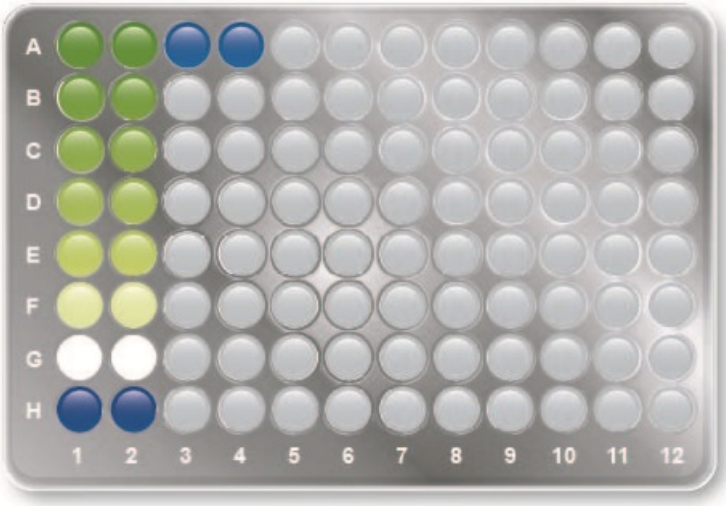
1. After collection, tissue samples should be flash frozen in liquid nitrogen. If analysis cannot be carried out immediately, store tissue at -80°C.
2. Grind frozen tissue to a fine powder under liquid nitrogen in a stainless steel mortar.
3. When liquid nitrogen has evaporated, weigh the frozen tissue and homogenize in 10 volumes of cold 5% TCA in a glass-Teflon tissue grinder.
4. Centrifuge at ≥600 x g to pellet the debris (~10 minutes).
5. Extract the supernatant with 3 volumes of water saturated ether.
6. Dry the aqueous extracts and reconstitute in at least 250 µL Assay Buffer 2 (to allow for duplicate measures).

Note: standards must be diluted in Assay Buffer 2, no Neutralizing Reagent is used.

13. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Return unused wells to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

Recommended plate layout



A1-2 - Standard #1
B1-2 - Standard #2
C1-2 - Standard #3
D1-2 - Standard #4
E1-2 - Standard #5
F1-2 - Standard #6 (Bo)
G1-2 - Blank Wells
H1-2 - Total Activity Wells (TA)

A3-4 - Non-Specific Binding Wells (NSB)

14. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**
- **Refer to the recommended plate layout in Section 13 before proceeding with the assay**

13. Prepare all reagents, working standards, and samples as directed in the previous sections.

14.2 If using samples prepared in 0.1M HCl. Add 50 µL of Neutralizing Reagent into each well except TA and Blank wells. Do not add Neutralizing Reagent for the other sample diluent options.

14.3 Add 100 µL of the appropriate standard diluent (Assay Buffer, 0.1M HCl, or non-conditioned culture meda) into NSB and B_O wells.

14.4 Add 50 µL of the appropriate standard diluent to the NSB wells.

14.5 Pipet 100 µL of Standards #1 through #5 to the bottom of the appropriate wells.

14.6 Add 100 µL of prepared samples and diluted samples to appropriate wells.

14.7 Add 50 µL of the Cyclic GMP Complete Alkaline Phosphatase Conjugate into each well except the TA and Blank wells.

14.8 Add 50 µL of the Cyclic GMP Complete antibody into each well except the Blank, TA and NSB wells.

Note: Every well used should be Green in color except the NSB well which should be Blue. The Blank and TA wells are empty at this point and have no color.

14.9 Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided.

14.10 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 Washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

14.11 Add 5µL of the Cyclic GMP Complete Alkaline Phosphatase Conjugate to the TA wells.

14.12 Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.

14.13 Add 50 µL Stop Solution into each well. The plate should be read immediately.

14.14 Read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm.

15. CALCULATIONS

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

1. 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

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

   Percent Bound = (Net OD/ Net Bo OD) x 100

3. Plot both the Percent Bound and the Net OD versus Concentration of Cyclic GMP for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting

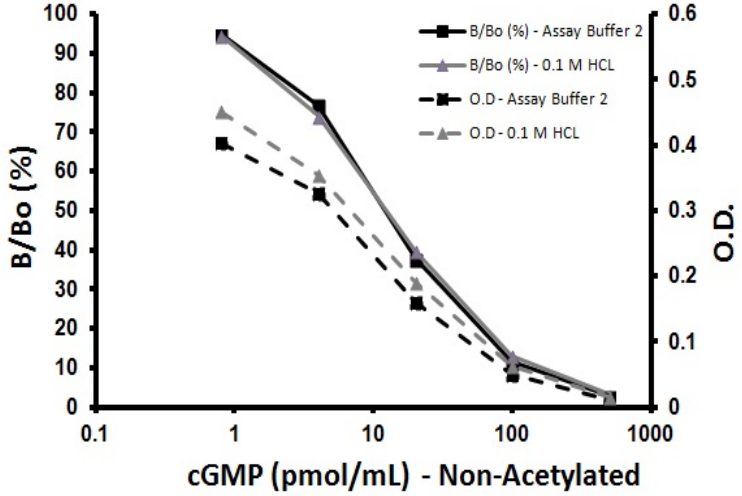
Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

To normalize for protein content, divide the resulting picomole per mL determinations (pmol/mL) by the total protein concentration (mg/mL) in each sample. This is expressed as pmol Cyclic GMP per mg of total protein.

16. TYPICAL DATA

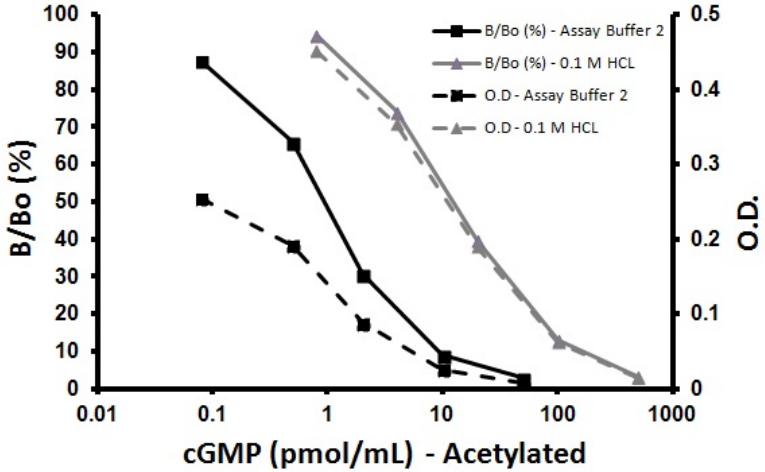
TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

Non-Acetylated Format:



Sample	Assay Buffer 2 Non-Acetylated			0.1 M HCL Non-Acetylated		
	Mean OD (-Blank)	% Bound	cGMP pmol/mL	Mean OD (-Blank)	% Bound	cGMP pmol/mL
Blank (mean)	(0.086)	-	-	(0.094)	-	-
TA	0.251	-	-	0.298	-	-
NSB	-0.001	0	-	0.000	0	-
Standard 1	0.012	2.8	500	0.015	3.1	500
Standard 2	0.049	11.5	100	0.062	13.0	100
Standard 3	0.160	37.7	20	0.190	39.7	20
Standard 4	0.327	76.9	4	0.354	74.1	4
Standard 5	0.403	94.8	0.8	0.451	94.4	0.8
B_O	0.425	100	0	0.478	100	0
Unknown 1	0.087	20.9	47	0.145	30.5	31
Unknown 2	0.367	86.4	2.2	0.344	71.9	4.5

Acetylated Format:



	Assay Buffer 2 Acetylated			0.1 M HCL Acetylated		
Sample	**Mean OD (-Blank)**	**% Bound**	**cGMP pmol/mL**	**Mean OD (-Blank)**	**% Bound**	**cGMP pmol/mL**
Blank (mean)	(0.091)	-	-	(0.123)	-	-
TA	0.254	-	-	0.335	-	-
NSB	-0.008	0	-	-0.002	0	-
Standard 1	0.008	2.8	50	0.008	2.8	50
Standard 2	0.026	9.0	10	0.032	11.4	10
Standard 3	0.088	30.5	2	0.098	34.9	2
Standard 4	0.191	65.9	0.5	0.220	78.3	0.4
Standard 5	0.254	87.6	0.08	0.279	99.3	0.08
B_O	0.290	100	0	0.281	100	0
Unknown 1	0.052	17.9	4.2	0.023	8.2	14
Unknown 2	0.086	29.8	2.1	0.055	19.9	4.4

17. TYPICAL SAMPLE VALUES

SENSITIVITY –

Assay Buffer 2

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as Bo (Standard #6), and comparing to the average optical density for sixteen (16) wells run with Standard #5. The detection limit was determined as the concentration of cGMP measured at two (2) standard deviations from the zero along the standard curve was determined to be 0.420 pmol/mL in the non-acetylated assay format and 0.043 pmol/mL in the acetylated assay format.

0.1M HCl

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as B_O, and comparing to the average optical density for sixteen (16) wells run with Standard 5. The detection limit was determined as the concentration of cGMP measured at two (2) standard deviations from the zero along the standard curve was determined to be 0.604 pmol/mL in the non-acetylated assay format and 0.059 pmol/mL in the acetylated assay format.

SAMPLE RECOVERY –

0.1M HCl should not be used to dilute culture supernates, serum, or saliva samples.

Recovery was determined by Cyclic GMP standard was spiked into the following matrices diluted with Assay Buffer 2 and measured in the kit. Mean recoveries are as follows:

Non-Acetylated Format:

Sample Type	Average % Recovery	Recommended Dilution
Tissue Culture Media	101.7	1:100
Human Serum	102.9	1:10
Human Saliva	101.3	≥1:10
Human Heparin Plasma	104.4	1:10
Human EDTA Plasma	115.0	≥1:10
Human Urine	97.7	≥1:100

Acetylated Format:

Sample Type	Average % Recovery	Recommended Dilution
Tissue Culture Media	95.8	Undiluted
Human EDTA Plasma	93.6	≥1:2

LINEARITY OF DILUTION –

A buffer sample containing Cyclic GMP was serially diluted 1:2 in the kit assay buffer and measured in the assay. The results are shown in the table below.

Non-Acetylated:

Dilution	Expected (pmol/mL)	Observed (pmol/mL)	Recovery (%)
Neat	-	45.6	-
1:2	22.8	24.6	108
1:4	11.4	13.5	118
1:8	5.7	6.3	111
1:16	2.9	3.0	103
1:32	1.4	1.9	135
1:64	0.71	0.96	135
1:128	0.36	0.38	106

Acetylated:

Dilution	Expected (pmol/mL)	Observed (pmol/mL)	Recovery (%)
Neat	-	3.4	-
1:2	1.7	1.7	100
1:4	0.85	0.89	105
1:8	0.43	0.42	98
1:16	0.21	0.19	90

A 0.1M HCl sample containing Cyclic GMP was serially diluted 1:2 in the 0.1M HCl diluent and measured in the assay. The results are shown in the table below.

Non-Acetylated:

Dilution	Expected (pmol/mL)	Observed (pmol/mL)	Recovery (%)
Neat	-	87.4	-
1:2	43.7	48.8	112
1:4	21.9	25.4	116
1:8	10.9	10.1	93
1:16	5.5	6.3	115
1:32	2.7	3.3	122

Acetylated:

Dilution	Expected (pmol/mL)	Observed (pmol/mL)	Recovery (%)
Neat	-	14.4	-
1:2	7.2	9.4	130
1:4	3.6	4.4	122
1:8	1.8	2.2	122
1:16	0.90	1.3	144
1:32	0.45	0.63	140
1:64	0.23	0.22	96

PRECISION –

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing Cyclic GMP Complete in a single assay.
Inter-assay precision was determined by measuring buffer controls of varying Cyclic GMP Complete concentrations in multiple assays over several days.

Non-Acetylated Format (Assay Buffer 2):

pmol/mL	Intra-Assay %CV
1.5	5.2
16.6	4.0
481	7.6

pmol/mL	Inter-Assay %CV
1.8	13.7
16.9	3.5
359	5.0

Acetylated Format (Assay Buffer 2):

pmol/mL	Intra-Assay %CV
0.54	6.5
1.5	4.6
6.8	4.5

pmol/mL	Inter-Assay %CV
0.70	5.9
2.0	6.2
8.6	6.8

Non-Acetylated Format (0.1M HCL):

pmol/mL	Intra-Assay %CV
1.6	4.4
9.9	7.9
115	6.6

pmol/mL	Inter-Assay %CV
2.1	6.0
8.5	9.9
92	6.9

Acetylated Format (0.1M HCL):

pmol/mL	Intra-Assay %CV
0.58	9.6
1.4	3.6
5.4	3.5

pmol/mL	Inter-Assay %CV
0.35	11
3.6	8.4
10	4.6

18. ASSAY SPECIFICITY

CROSS REACTIVITY –

The cross reaction of the antibody calculated at 50% is:

Compound	Cross Reavitivity
Cyclic GMP	100
GMP	<0.001
GTP	<0.001
cAMP	<0.001
AMP	<0.001
ATP	<0.001
cUMP	<0.001
CTP	<0.001

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed

20. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)