Product Manual

Cyclic GMP Complete

Catalog #: ADI-900-164

96-well Kit

For use with cells, tissue, saliva, serum, plasma, urine and culture supernates

For the latest product information, including support documentation, visit us online:
www.enzolifesciences.com

Technical Support (US): 800-942-0430
Technical Support (EU): +41 61 926 8989

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research use only. Not for use in diagnostic procedures. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON- INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

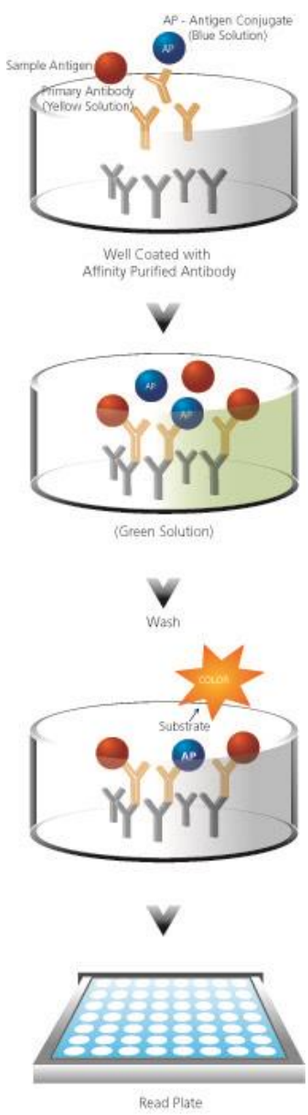
FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

All reagents, except standard and conjugate, should be stored at 4˚C. Store standard and conjugate at -20˚C

For proper performance, use the insert provided with each individual kit received.

Check our website for additional protocols, technical notes and FAQs.

TABLE OF CONTENTS

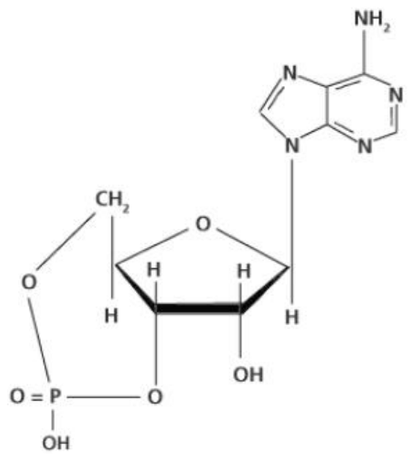


INTRODUCTION

The cGMP complete Enzyme-linked Immunosorbent Assay (ELISA) kit is a competitive immunoassay for the quantitative determination of cyclic GMP in cells and tissue treated with 0.1M HCl, in addition to culture supernatants, saliva, and serum. The optional acetylated assay format provides an approximate 10-fold increase in sensitivity and is ideal for samples with extremely low levels of cGMP. If expected levels of cGMP are unknown, the investigator may evaluate a few samples in the non-acetylated format in order to determine if higher sensitivity is required.

Guanosine 3',5'-cyclic monophosphate (cyclic GMP; cGMP) was identified in 1963[1]. 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 cGMP levels[2,3]. Stimulators of guanylate cyclase such as the vasodilators niroprusside, nitroglycerin, sodium nitrate, and nitric oxide (NO) also stimulate cGMP levels[4]. Peptides, such as atrial natriuretic peptide (ANP) that relax smooth muscle also increase cGMP concentrations[5]. cGMP has been confirmed as a second messenger for ANP[6]. NO can be synthesized from L-arginine and diffuse through cell membranes[7,8]. The interaction of NO with guanylate cyclase allows cGMP to act as a third messenger in some cells[9].

cyclic GMP



PRINCIPLE

1. Standards and samples are added to wells coated with a GxR IgG antibody. A blue solution of cGMP conjugated to alkaline phosphatase is then added, followed by a yellow solution of rabbit polyclonal antibody to cGMP.
2. During a simultaneous incubation at room temperature the antibody binds, in a competitive manner, the cGMP in the sample or conjugate. The plate is washed, leaving only bound cGMP.
3. pNpp substrate solution is added. The substrate generates a yellow color when catalyzed by the alkaline phosphatase on the cGMP conjugate.
4. Stop solution is added. The yellow color is read at 405nm. The amount of signal is indirectly proportional to the amount of cGMP in the sample.

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

HCl is caustic. Keep tightly capped.

The standard should be handled with care due to the known and unknown effects of the antigen.

Triethylamine and acetic anhydride are lachrymators. Caution- corrosive, flammable, and harmful vapor.

Avoid contamination by endogenous alkaline phosphatase. Do not expose reagents or supplies to bare skin.

Activity of conjugate is affected by concentrations of chelators > 10mM (such as EDTA and EGTA).

Stop solution is caustic. Keep tightly capped.

MATERIALS SUPPLIED

1. **Assay Buffer 2 Concentrate**
   27 mL, Product No. 80-0055
   Sodium acetate buffer containing proteins and sodium azide
2. **0.1M HCl**
   27 mL, Product No. 80-0080
   0.1M hydrochloric acid in water
3. **cyclic GMP Standard**
   0.5 mL, Product No. 80-0153
   A solution of 5,000 pmol/mL cGMP
4. **Acetylation Kit**
   2 vials, Product No. 950-001
   a. **Triethylamine**
      2 mL, Product No. 80-0063
   b. **Acetic Anhydride**
      1 mL, Product No. 80-0064
5. **Goat anti-Rabbit IgG Microtiter Plate**
   One plate of 96 wells, Product No. 80-0060
   A clear plate of break-apart strips coated with a goat anti-rabbit polyclonal antibody
6. **Neutralizing Reagent**
   5 mL, Product No. 80-1475
7. **cGMP Antibody**
   5 mL, Product No. 80-0152
   A yellow solution of rabbit polyclonal antibody to cGMP
8. **cGMP Conjugate**
   5 mL, Product No. 80-0151
   A blue solution of cGMP conjugated to alkaline phosphatase
9. **Wash Buffer Concentrate**
   27 mL, Product No. 80-1286
   Tris buffered saline containing detergents
10. **pNpp Substrate**
    20 mL, Product No. 80-0075
    A solution of p-nitrophenyl phosphate
11. **Stop Solution**
    5 mL, Product No. 80-0247
    A solution of trisodium phosphate in water
12. **cGMP Complete Assay Layout Sheet**
    1 each, Product No. 30-0247
13. **Plate Sealer**
    1 each, Product No. 30-0012

Reagents require separate storage conditions.

STORAGE

All components of this kit, **except the Conjugate and Standard**, are stable at 4°C until the kit's expiration date. Upon receipt, store the Conjugate and Standard at -20°C. Recommended storage temperatures do not necessarily reflect shipping conditions.

MATERIALS NEEDED BUT NOT SUPPLIED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 50 µL and 200 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Microplate shaker
7. Lint-free paper toweling for blotting
8. Microplate reader capable of reading at 405 nm

REAGENT PREPARATION

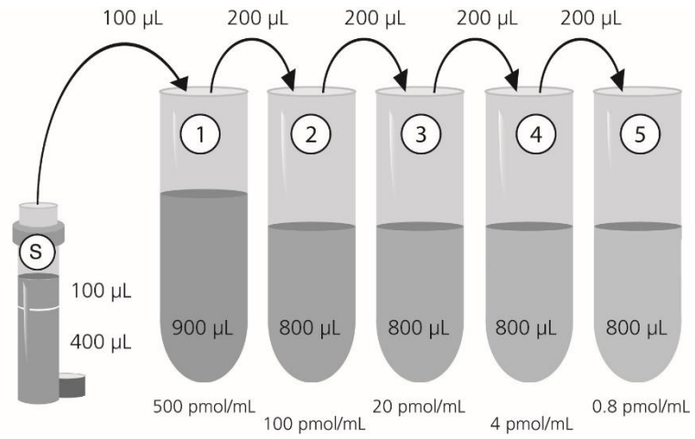
Glass or poly-propylene tubes may be used for standard preparation. Avoid poly-styrene.

1. **Wash Buffer**

   Prepare the wash buffer by diluting 5 mL of the supplied Wash Buffer Concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **cGMP Standard, non-acetylated format**

   Three diluent options are available for the preparation of the standard curve, NSB, and Bo wells. For cell lysates and tissue samples prepared in 0.1M HCl, use the supplied 0.1M HCl as the standard diluent. Use Assay Buffer 2 for the standard diluent when the sample is serum, plasma, urine, or saliva. For culture supernatants, use the same non-conditioned media for the standard diluent.



   Allow the 5,000 pmol/mL standard stock to warm to room temperature. Label five 12mm x 75mm tubes #1 through #5. Pipet 900 µL of the appropriate sample diluent into tube #1. Pipet 800 µL of the appropriate sample diluent into tubes #2 through #5. Add 100 µL of the 5,000 pmol/mL standard stock into tube #1 and vortex thoroughly. Add 200 µL of tube #1 to tube #2 and vortex thoroughly. Add 200 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #5.

   Diluted standards should be used within 60 minutes of preparation. The concentrations of cGMP in the tubes are labeled above.

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.

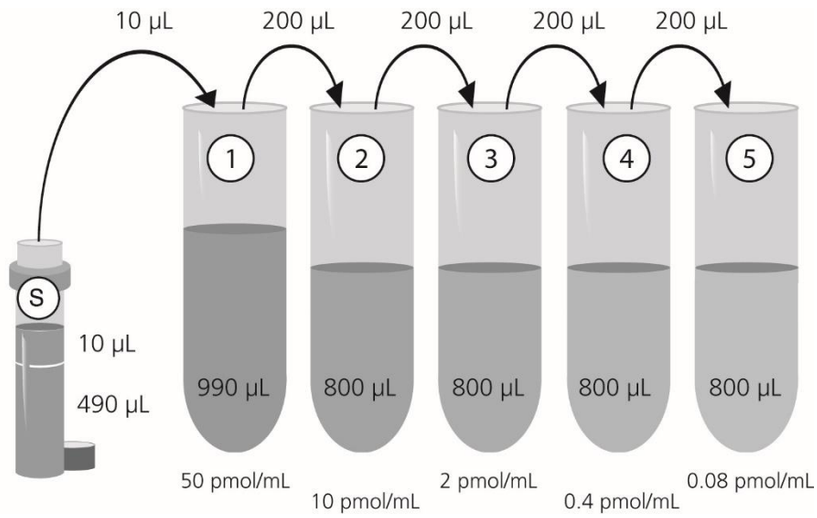
Triethylamine and acetic anhydride are lachrymators. Caution-corrosive, flammable, and harmful vapor.

Discard any unused portion of the Acetylating Reagent.

4. **cGMP Standard, acetylated format (optional)**

Three diluent options are available for the preparation of the standard curve, NSB, and Bo wells. For cell lysates and tissue samples prepared in 0.1M HCl, use the supplied 0.1M HCl as the standard diluent. Use Assay Buffer 2 for the standard diluent when the sample is serum or saliva. For culture supernatants, use the same non-conditioned media for the standard diluent.

Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.



Allow the 5,000 pmol/mL standard stock to warm to room temperature. Label five 12mm x 75mm tubes #1 through #5. Pipet 990 µL of the appropriate sample diluent into tube #1. Pipet 800 µL of the appropriate sample diluent into tubes #2 through #5. Add 10 µL of the 5,000 pmol/mL standard stock into tube #1 and vortex thoroughly. Add 200 µL of tube #1 to tube #2 and vortex thoroughly. Add 200 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #5.

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

Label one 12mm x 75mm tube as the Bo/NSB tube. Pipet 1 mL of the appropriate standard diluent into this tube. Add 50 µL of the Acetylating Reagent to the Bo/NSB tube and use in Steps 2 and 3 of the Assay Procedure.

The acetylated standards should be used within 30 minutes of preparation.The concentrations of cGMP in the tubes are labeled above.

SAMPLE HANDLING

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Samples must be stored at or below -20˚C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

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. Recommended treatment protocols follow. Samples containing rabbit IgG will interfere with the assay. EDTA plasma may precipitate during acetylation.

Biological fluids, such as serum, plasma, urine, and saliva, should be diluted in Assay Buffer 2 and run directly in the assay. Culture Supernatants should also be diluted in Assay Buffer 2 and the same non-conditioned media diluted with the assay buffer should be used as the standard diluent.

Please note that some samples may contain high levels of cGMP and additional dilution may be required. Samples with low levels of cGMP may be assayed in the acetylated format or the samples may be concentrated.

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.

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.
2. Incubate the cells in 0.1M HCl for 10 minutes at room temperature. Inspect the cells under a microscope to ensure uniform lysis. Continue incubating for an additional 10 minutes, if necessary.

3. Centrifuge ≥600 x g to pellet the cellular debris.
4. 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.

Protocol for Tissue Samples

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.

SAMPLE RECOVERIES

cGMP standard was spiked into the following matrices diluted with Assay Buffer 2 and measured in the kit. The results were as follows:

	Non-Acetylated Format		Acetylated Format	
Sample	**% Recovery**	**Recommended Dilution**	**% Recovery**	**Recommended Dilution**
Tissue Culture Media	101.7%	1:100	95.8	undiluted
Human Saliva	102.9%	1:10		
Human Serum	101.3%	≧ 1:10		Not Recommended
Human Heparin Plasma	104.4%	1:10		
Human EDTA Plasma	115.0%	≥ 1:10	93.6	≥ 1:2
Human Urine	97.7%	≥ 1:100		

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

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

Note: If the **acetylated format** of the assay is to be run, all standards, samples, and the diluent for the NSB and Bo wells must be acetylated as per the instructions in the Reagent Preparation section. Acetylated standards and samples must be used within 30 minutes.

1. If using samples prepared in 0.1M HCl, pipet 50 µL of Neutralizing Reagent into each well except the Total Activity (TA) and Blank wells. Do not add Neutralizing Reagent for the other sample diluent options.
2. Pipet 100 µL of the appropriate standard diluent (Assay Buffer 2, 0.1M HCl, or non-conditioned culture media) into the NSB (non-specific binding) and Bo (0 pmol/mL standard) wells.
3. Add 50 µL of the appropriate standard diluent to the NSB wells.
4. Pipet 100 µL of Standards #1 through #5 to the bottom of the appropriate wells.
5. Pipet 100 µL of the samples to the bottom of the appropriate wells.
6. Pipet 50 µL of the blue conjugate into each well except the TA and Blank wells.
7. Pipet 50 µL of the yellow antibody into each well except the Blank, TA, and NSB wells.

   Note: Every well used should be green in color except the NSB wells which should be blue. The Blank and TA wells are empty at this point and have no color.
8. Seal the plate. Incubate for 2 hours on a plate shaker (~500 rpm) at room temperature.
9. Empty the contents of the wells and wash by adding 400 µL of wash buffer to every well. Repeat 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells by inverting the plate and firmly tapping it on a lint-free paper towel to remove any remaining wash buffer.
10. Pipet 5µL of the blue conjugate to the TA wells.
11. Add 200 µL of the substrate solution into each well.
12. Incubate for 1 hour at room temperature without shaking.
13. Pipet 50 µL stop solution into each well.

14. After blanking the plate reader against the substrate blank, read optical density at 405 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of cGMP in samples. We recommend that the data be handled by an immunoassay software package such as Assay Blaster Data Analysis Software (Product no. ADI-28-0002) utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average 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:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

3. Plot the Percent Bound (B/Bo) versus concentration of cGMP for the standards. Approximate a straight line through the points. The concentration of cGMP of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re- analyzed using a different dilution.

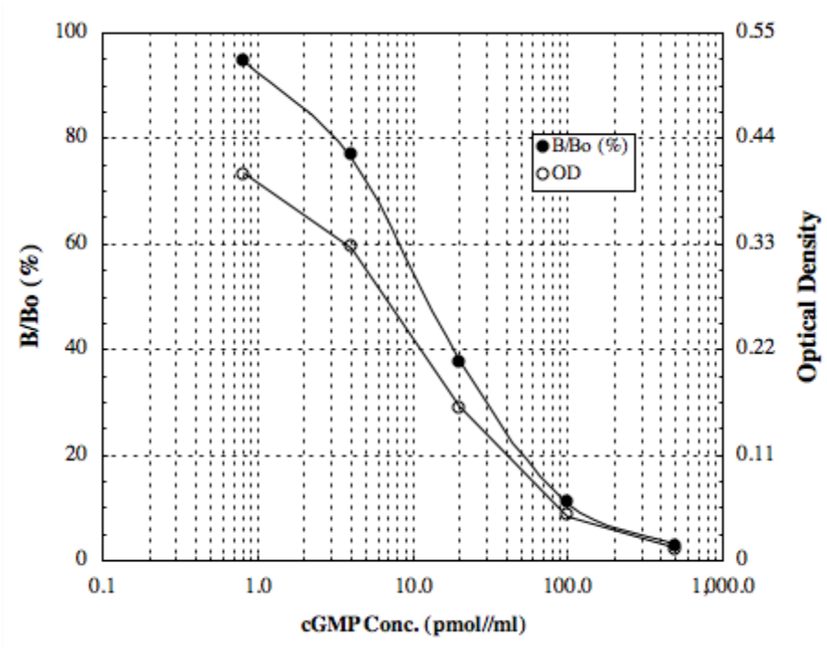
To normalize for protein content, divide the resulting picomole per mL determinations (pmol/mL) by the total protein concentration (mg/mL) in each sample. This is expressed as pmol cGMP per mg of total protein.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results from another assay.

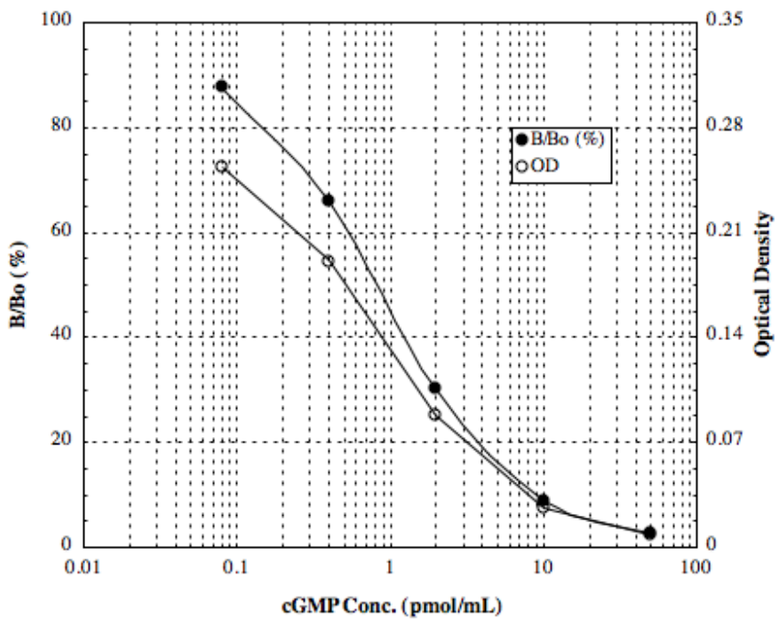
Non-acetylated assay format in Assay Buffer 2

Sample	Average Net OD	Percent Bound	cGMP (pmol/mL)
Blank (mean)	(0.086)	---	---
TA	0.251	---	---
NSB	-0.001	---	---
Bo	0.425	100%	0
S1	0.012	2.8%	500
S2	0.049	11.5%	100
S3	0.160	37.7%`	20
S4	0.327	76.9%	4
S5	0.403	94.8%	0.8
Unknown 1	0.087	20.9%	47
Unknown 2	0.367	86.4%	2.2



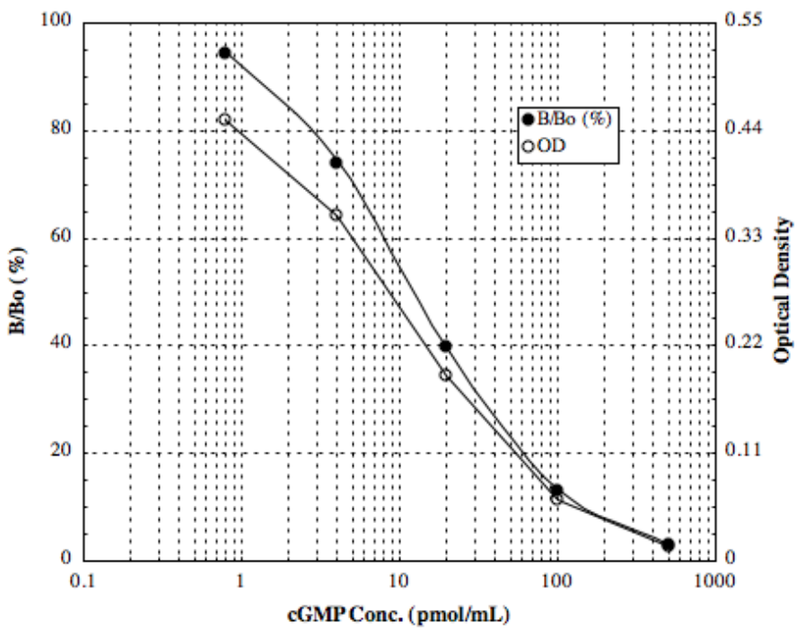
Acetylated assay format in Assay Buffer 2

Sample	Average Net OD	Percent Bound	cGMP (pmol/mL)
Blank (mean)	(0.091)	---	---
TA	0.254	---	---
NSB	-0.008	---	---
Bo	0.290	100%	0
S1	0.008	2.8%	50
S2	0.026	9.0%	10
S3	0.088	30.5%	2
S4	0.191	65.9%	0.5
S5	0.254	87.6%	0.08
Unknown 1	0.052	17.9%	4.2
Unknown 2	0.086	29.8%	2.1



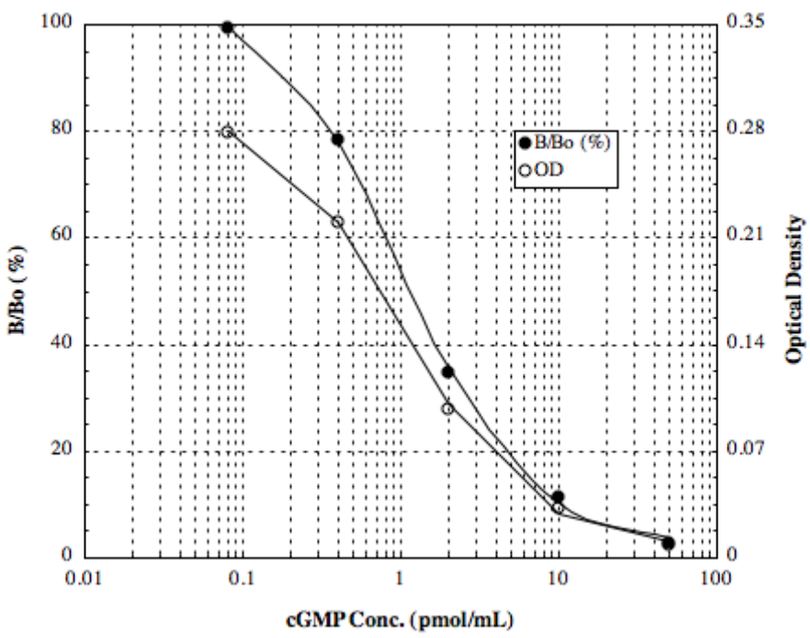
Non-acetylated assay format in 0.1 M HCl

Sample	Average Net OD	Percent Bound	cGMP (pmol/mL)
Blank (mean)	(0.094)	---	---
TA	0.298	---	---
NSB	0.000	---	---
Bo	0.478	100%	0
S1	0.015	3.1%	500
S2	0.062	13.0%	100
S3	0.190	39.7%	20
S4	0.354	74.1%	4
S5	0.451	94.4%	0.8
Unknown 1	0.145	30.5%	31
Unknown 2	0.344	71.9%	4.5



Acetylated assay format in 0.1 M HCl

Sample	Average Net OD	Percent Bound	cGMP (pmol/mL)
Blank (mean)	(0.123)	---	---
TA	0.335	---	---
NSB	-0.002	0%	---
Bo	0.281	100%	0
S1	0.008	2.8%	50
S2	0.032	11.4%	10
S3	0.098	34.9%	2
S4	0.220	78.3%	0.4
S5	0.279	99.3%	0.08
Unknown 1	0.023	8.2%	14
Unknown 2	0.055	19.9%	4.4



PERFORMANCE CHARACTERISTICS

Specificity

The cross reactivities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration of ten times the high standard. These samples were then measured in the assay.

Compound	Cross Reactivity
cGMP	100%
GMP	<0.001%
GTP	<0.001%
cAMP	<0.001%
AMP	<0.001%
ATP	<0.001%
cUMP	<0.001%
CTP	<0.001%

Sensitivity

Assay Buffer 2

The sensitivity of the assay, defined as the concentration of cGMP measured at 2 standard deviations from the mean of 16 zeros 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

The sensitivity of the assay, defined as the concentration of cGMP measured at 2 standard deviations from the mean of 16 zeros 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.

Linearity

A buffer sample containing cGMP 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 cGMP 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 cGMP in a single assay.

Non-Acetylated Format

In Assay Buffer 2		In 0.1M HCl	
pmol/mL	**%CV**	**pmol/mL**	**%CV**
1.5	5.2	1.6	4.4
16.6	4.0	9.9	7.9
481	7.6	115	6.6

Acetylated Format

In Assay Buffer 2		In 0.1M HCl	
pmol/mL	**%CV**	**pmol/mL**	**%CV**
0.54	6.5	0.58	9.6
1.5	4.6	1.4	3.6
6.8	4.5	5.4	3.5

Inter-assay precision was determined by measuring buffer controls of varying cGMP concentrations in multiple assays over several days.

Non-Acetylated Format

In Assay Buffer 2		In 0.1M HCl	
pmol/mL	**%CV**	**pmol/mL**	**%CV**
1.8	13.7	2.1	6.0
16.9	3.5	8.5	9.9
359	5.0	92	6.9

Acetylated Format

In Assay Buffer 2		In 0.1M HCl	
pmol/mL	**%CV**	**pmol/mL**	**%CV**
0.70	5.9	0.35	11
2.0	6.2	3.6	8.4
8.6	6.8	10	4.6

REFERENCES

1. E.W. Sutherland, G.A. Robison, and R.W. Butcher, Circulation, (1968) 37: 279.
2. T.W. Rall, et al., J. Biol. Chem., (1957) 224: 463.
3. T.W. Cook, et al., J. Am. Chem. Soc., (1957), 79: 3607.
4. E.W. Sutherland, and T.W. Rall, J. Am. Chem. Soc., (1957) 79: 3608.
5. D. Lipkin, et al., J. Am. Chem. Soc., (1959) 81: 6198.
6. D. Chabardes, et al., J. Clin. Invest., (1980) 65: 439.
7. V. Grill, and E. Cerasi, J. Biol. Chem., (1974) 249: 41961.
8. R.C. Haynes, J. Biol. Chem., (1958), 233: 1220.
9. A. Szentivanyi, J. Allergy, (1968) 42: 203.
10. P. Hamet, et al, Adv. Cycl. Nucl. Res., (1983) 15: 11.
11. M. Plaut, et al, Adv. Cycl. Nucl. Res., (1983) 12: 161.
12. J.H. Exton, Adv. Cycl. Nucl. Res., (1983) 12: 319.

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:

www.enzolifesciences.com

Document Number: 25-0618 Revision: 090519