Product Manual

Direct cGMP ELISA kit

Catalog #: ADI-900-014

96 Well Kit

For use with cells and tissue samples

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.

Product Manual

TABLE OF CONTENTS

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

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

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

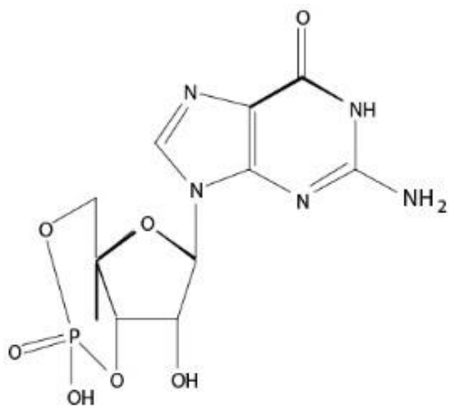
Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

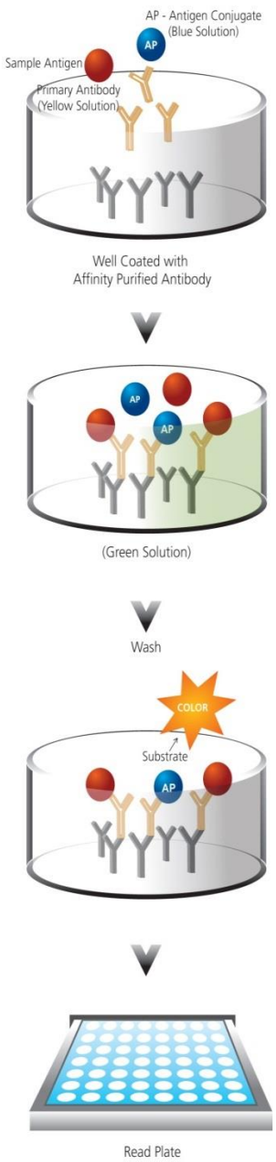
The cyclic GMP Enzyme-Linked Immunosorbent Assay (ELISA) kit is a competitive immunoassay for the quantitative determination of cyclic GMP in samples treated with 0.1 M HCl. The acetylated assay format provides an increase in sensitivity and is ideal for samples with extremely low levels of cGMP. The optional overnight acetylated assay format allows super-sensitive detection of cGMP. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Guanosine 3', 5'-cyclic monophosphate (cyclic GMP; cGMP) 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[3]. 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[4,5]. Stimulators of guanylate cyclase such as the vasodilators nitroprusside, nitroglycerin, sodium nitrate and nitric oxide (NO) also stimulate cGMP levels[6]. Peptides, such as atrial natriuretic peptide (ANP) that relax smooth muscle also increase cGMP concentrations[7]. cGMP has been confirmed as a second messenger for ANP[8]. NO can be synthesized from L-arginine and diffuse through cell membranes[9,10]. The interaction of NO with guanylate cyclase allows cGMP to act as a third messenger in some cells[11].

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.

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

MATERIALS SUPPLIED

1. **Goat anti-Rabbit IgG Microtiter Plate, 1 Plate of 96 Wells, Catalog No. 80-0060**

   A plate using break-apart strips coated with goat antibody specific to rabbit IgG.

2. **Cyclic GMP Standard, 0.5 mL, Catalog No. 80-0153**

   A solution of 5,000 pmol/mL cGMP.

3. **cGMP ELISA Antibody, 5 mL, Catalog No. 80-0152**

   A yellow solution of a rabbit polyclonal antibody to cGMP.

4. **cGMP Conjugate, 5 mL, Catalog No. 80-0151**

   A blue solution of alkaline phosphatase conjugated with cGMP.

5. **0.1 M HCl, 27 mL, Catalog No. 80-0080**

   0.1 M hydrochloric acid in water.

6. **Acetylation Kit, 2 vials, Catalog No. 950-001**
   1. **Triethylamine, 2 mL, Catalog No. 80-0063**
   2. **Acetic Anhydride, 1 mL, Catalog No. 80-0064**

7. **Neutralizing Reagent, 5 mL, Catalog No. 80-1475**

8. **Wash Buffer Concentrate, 27 mL, Catalog No. 80-1286**

   Tris buffered saline containing detergents.

9. **pNpp Substrate, 20 mL, Catalog No. 80-0075**

   A solution of p-nitrophenyl phosphate in buffer. Ready to use.

10. **Stop Solution, 5 mL, Catalog No. 80-0247**

    A solution of trisodium phosphate in water. Keep tightly capped.

11. **Direct cGMP Assay Layout Sheet, 1 each, Catalog No. 30-0033**

12. **Plate Sealer, 1 each, Catalog No. 30-0012**

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

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).

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

Protect substrate from prolonged exposure to light.

HCl and stop solution are caustic. Keep tightly capped.

Reagents require separate storage conditions.

STORAGE

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

OTHER MATERIALS NEEDED

Avoid repeated freeze/ thaw cycles of the conjugate.

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipets for dispensing 50 µL and 200 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
9. A centrifuge (for sample preparation).
10. Triton X-100 or equivalent (optional for sample preparation).
11. Liquid nitrogen, stainless steel mortar & pestle (optional – for tissue samples).

Sample handling procedures should be completed prior to reagent preparation.

SAMPLE HANDLING

Treatment of cells and tissue with the supplied 0.1 M 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. EDTA plasma may precipitate during acetylation. **Samples containing rabbit IgG will interfere with the assay.**

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.

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 or overnight acetylated format, or the samples may be concentrated. The end user **must verify** that the recommended dilutions are appropriate for their samples.

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 0.1 M HCl for enhanced lysis. If Triton X-100 is added to samples it should also be added to the standard dilution at the same percentage, as a modest increase in optical density may occur.

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

1. Pellet **suspension cells** and aspirate the media. Treat cells with 0.1 M HCl. A general starting concentration of 1 x 10^6 cells per mL of 0.1 M HCl is recommended. Remove the media from **adherent cells** and add enough 0.1 M HCl to cover the bottom of the plate. Avoid over-diluting the sample with an excessive volume of HCl.
2. Incubate the cells in 0.1 M HCl for 10 minutes at room temperature.
3. Inspect the cells under a microscope to ensure uniform lysis. Continue incubating for an additional 10 minutes, if necessary.
4. Centrifuge ≥600 x g to pellet the cellular debris.
5. The supernatant may be assayed immediately or stored frozen, ideally at -70°C or below, for later analysis.

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 -70˚C or below.
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 0.1 M HCl (e.g., 0.1g 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 0.1 M HCl provided and run directly in the assay or stored frozen, ideally at -70°C or below, for later analysis.

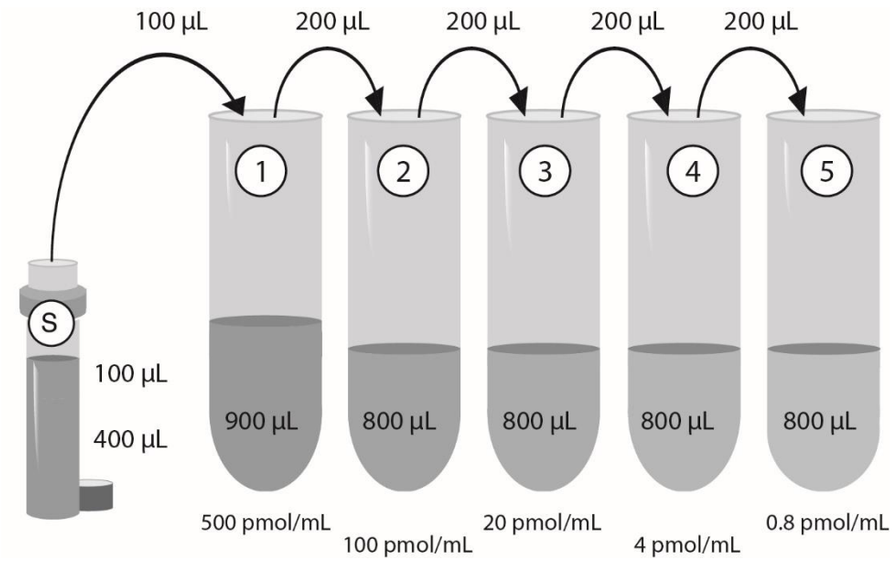
Biological fluids (e.g. serum, plasma, saliva) and culture supernates should be used in the cyclic GMP ELISA Kit (Cat. #ADI-900-013, ADI-901-013) or the cyclic GMP Complete ELISA Kit (Cat. #ADI-900-164, ADI-901-164).

REAGENT PREPARATION

1. cGMP Standard - Non-Acetylated Format

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

Allow the 5,000 pmol/mL cGMP standard stock to warm to room temperature. Label five 12 x 75mm tubes #1 through #5. Pipet 900 µL of the 0.1 M HCl into tube #1. Pipet 800 µL of the 0.1 M HCl into tubes #2 through #5. Add 100 µL of the 5,000 pmol/mL standard stock to tube #1. Vortex thoroughly. Add 200 µL of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #5.



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

The concentration of cGMP in the tubes are labeled above.

Diluted standards should be used within 60 minutes of preparation.

2. Acetylation Reagent (optional)

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

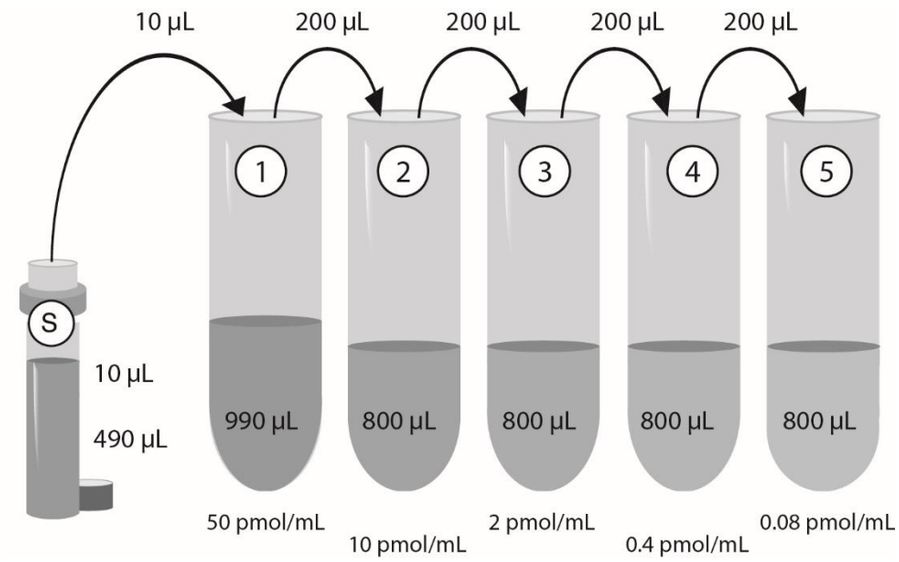
Prepare the Acetylation Reagent by adding 0.5 mL of acetic anhydride to 1 mL triethylamine and vortex thoroughly. Note that this volume is sufficient to add to up to 30 mL of diluted standard and samples. Use the prepared reagent within 60 minutes of preparation. Discard any unused portion of the Acetylation Reagent.

3. cGMP Standard - Acetylated Format

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

Allow the 5,000 pmol/mL cGMP standard stock to warm to room temperature. Label five 12 x 75 mm tubes #1 through #5. Pipet 990 µL 0.1M HCl into tube #1 and 800 µL 0.1 M HCl into tubes #2-5. Add 10 µL of the 5,000 pmol/mL standard to tube #1. Vortex thoroughly. Add 200 µL of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #5.

Acetylate all standards and samples by adding 10 µL of the Acetylation Reagent for each 200 µl of standard or sample. Add the reagent directly to the samples and vortex thoroughly for 2 seconds immediately after the addition of the Acetylation Reagent.



The concentration of cGMP in the tubes are labeled above.

Label one 12 x 17 mm tube as the Zero Standard/NSB tube. Pipet 1 mL of the 0.1 M HCl into this tube. Add 50 µL of the Acetylating Reagent to the Zero Standard/NSB tube, vortex immediately, and use this for Steps 2 and 5 in the Assay Procedure. **Failure to acetylate the NSB and Zero Standard will result in inaccurate B/Bo values.**

Use the acetylated standards and samples within 30 minutes of preparation.

4. **Wash Buffer**

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

ASSAY PROCEDURE

All standards and samples should be run in duplicate.

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.

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

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.

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

1. Pipet 50 µL of the Neutralizing Reagent into each well, except the Total Activity (TA) and Blank wells.
2. Pipet 100 µL of the 0.1 M HCl into the NSB (non-specific binding) and the Bo (0 pmol/mL Standard) wells.
3. Pipet an additional 50 µL of 0.1M HCl into the NSB wells.
4. Pipet 100 µL of Standards into the appropriate wells.
5. Pipet 100 µL of the Samples into the appropriate wells.
6. Pipet 50 µL of blue Conjugate into each well except the TA and Blank wells.
7. Pipet 50 µL of 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.

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

8. Incubate the plate at room temperature for 2 hours on a plate shaker at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired. If using the optional Overnight Acetylated Format, incubate for 18-24 hours at 4°C tightly covered with the plate sealer provided.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 washes.

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

10. 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.
11. Add 5 µL of the blue Conjugate to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.
13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.

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

CALCULATION OF RESULTS

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

Several options are available for the calculation of the concentration of cGMP in the samples. We recommend that the data be handled by an immunoassay software package utilizing a weighted 4 parameter logistic curve fitting program. If this type of data reduction software is not readily available, the concentration of cGMP can be calculated as follows:

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

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

2. Plot the Net OD versus Concentration of cGMP for the standards. Sample concentrations of cGMP may be calculated by interpolation off of the standard curve using Net OD values.

3. Alternatively, calculate the binding of each standard and sample as a percentage of the maximum binding wells (Bo), using the formula below:

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

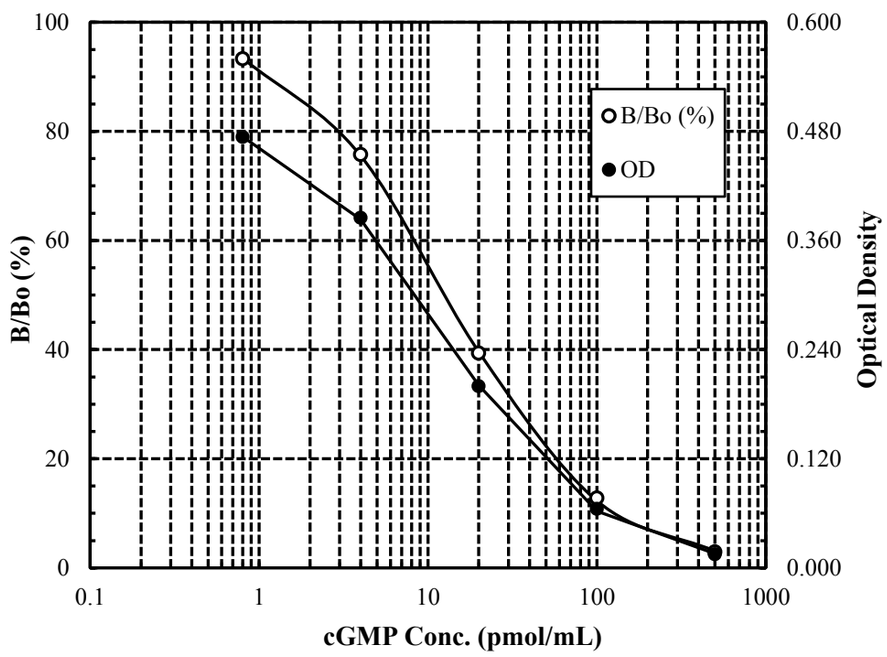
4. Plot the Percent Bound (B/Bo) versus Concentration of cGMP for the standards. Sample concentrations of cGMP may be calculated by interpolation off of the standard curve using Percent Bound values.

To normalize samples 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 aspmol cGMP per mg total protein.

TYPICAL RESULTS

The results shown below are for illustration only and **should not** be used to calculate results from another assay; each user must run a standard curve for each assay and version used.

Non-Acetylated Format			
Sample	**Ave Net OD**	**% B/Bo**	**cGMP (pmol/mL)**
Blank	(0.083)	---	---
TA	0.326	---	---
NSB	-0.001	-0.3%	---
Bo	0.508	100%	**0**
S1	0.015	2.9%	**500**
S2	0.065	12.7%	**100**
S3	0.200	39.3%	**20**
S4	0.385	75.8%	**4**
S5	0.474	93.3%	**0.8**
Control1	0.070	13.7%	**87.9**
Control2	0.173	34.0%	**25.7**



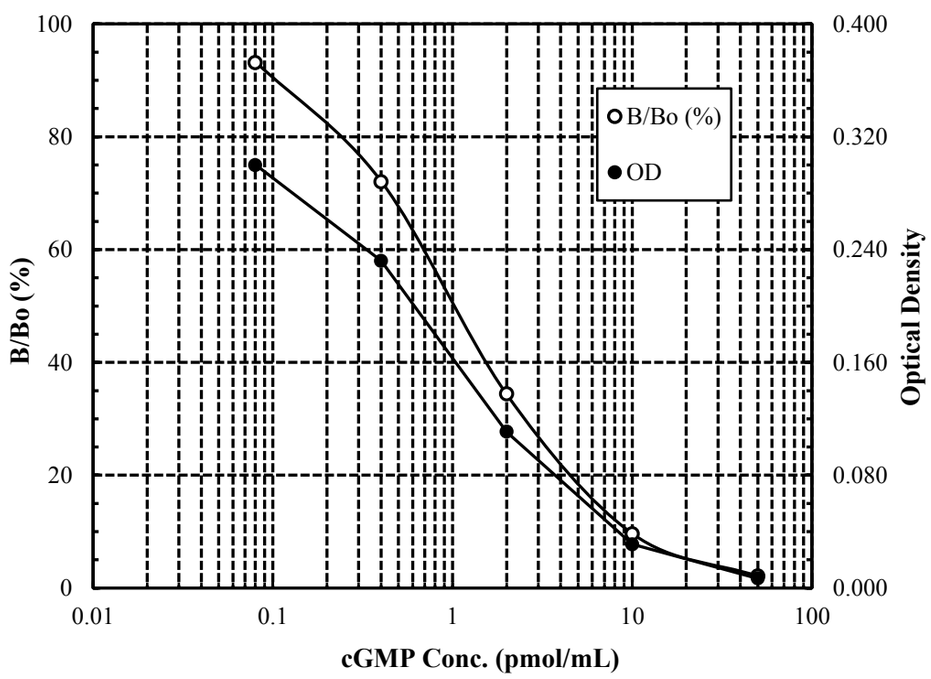
Quality of Fit = 1.000 (Calculated from 4 parameter logistic curve fit)

20% Intercept = 54.6 pmol/mL

50% Intercept = 12.9 pmol/mL

80% Intercept = 3.1 pmol/mL

Acetylated 2 Hour Format			
Sample	**Ave Net OD**	**% B/Bo**	**cGMP (pmol/mL)**
Blank	(0.086)	---	---
TA	0.322	---	---
NSB	-0.005	-1.6%	---
Bo	0.322	100%	**0**
S1	0.007	2.2%	**50**
S2	0.031	9.5%	**10**
S3	0.111	34.3%	**2**
S4	0.232	71.9%	**0.4**
S5	0.300	93.2%	**0.08**
Control3	0.038	11.8%	**7.93**
Control4	0.093	28.9%	**2.57**



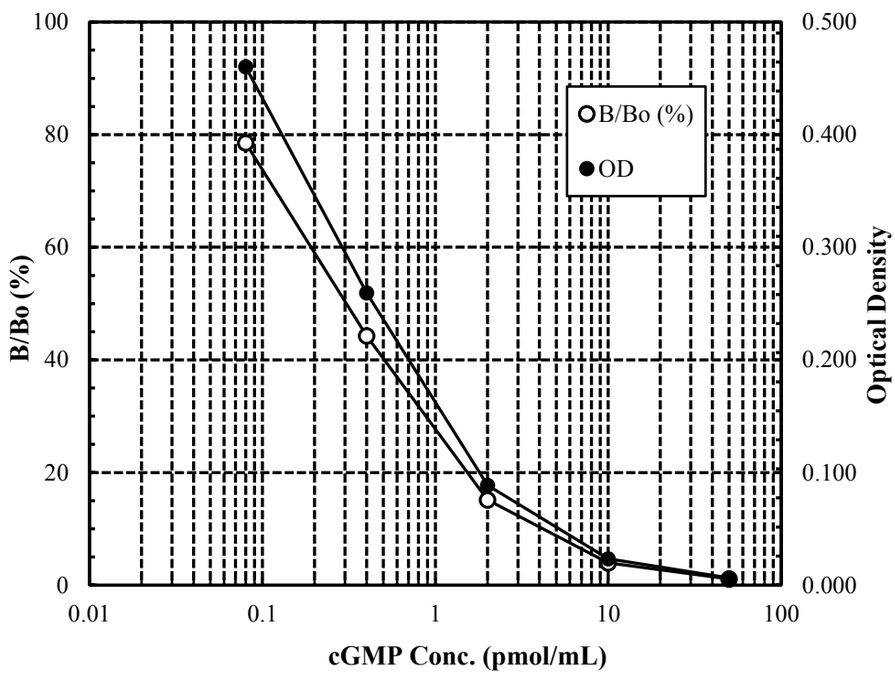
Quality of Fit = 1.000 (Calculated from 4 parameter logistic curve fit)

20% Intercept = 4.21 pmol/mL

50% Intercept = 1.03 pmol/mL

80% Intercept = 0.26 pmol/mL

Acetylated Overnight Format			
Sample	**Ave Net OD**	**% B/Bo**	**cGMP (pmol/mL)**
Blank	(0.084)	---	---
TA	0.342	---	---
NSB	-0.005	-0.8%	---
Bo	0.584	100%	**0**
S1	0.005	0.9%	**50**
S2	0.026	4.4%	**10**
S3	0.088	15.0%	**2**
S4	0.260	44.5%	**0.4**
S5	0.461	78.9%	**0.08**
Control3	0.022	3.8%	**10.71**
Control4	0.071	12.1%	**2.66**



Quality of Fit = 1.000 (Calculated from 4 parameter logistic curve fit)

20% Intercept = 1.40 pmol/mL

50% Intercept = 0.32 pmol/mL

80% Intercept = 0.08 pmol/mL

SAMPLE RECOVERIES

Please refer to pages 5-8 for Sample Handling recommendations and Standard preparation.

cGMP concentrations were measured in 0.1 M HCl, which is reflective of the lysate or tissue supernatant after sample treatment. cGMP was spiked into the undiluted sample, which was then serially diluted with the kit 0.1 M HCl and then tested in the kit. The following results were obtained:

	Non-Acetylated Format		Acetylated 2-Hr Format		Acetylated Overnight Format	
Sample	**% Recovery**	**Recom-mended Dilution***	**% Recovery**	**Recom-mended Dilution***	**% Recovery**	**Recom-mended Dilution***
0.1M HCl	101.7	None	115.6	None	108.1	None

* See Sample Handling instructions on page 5 for details.

PERFORMANCE CHARACTERISTICS

Sensitivity

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 format, 0.059 pmol/mL in the acetylated 2 hour format, and 0.025 pmol/mL in the acetylated overnight format.

Linearity

A 0.1 M HCl sample containing cGMP was serially diluted 1:2 in the 0.1 M HCl kit diluent and measured in the non-acetylated assay format. A separate 0.1 M HCl sample was serially diluted and measured in both acetylated assay formats. The results are shown below.

Non-Acetylated Format in 0.1 M HCl			
Dilution	**Expected (pmol/mL)**	**Observed (pmol/mL)**	**Recovery (%)**
Neat	---	240.6	---
1:2	120.3	115.5	96%
1:4	60.1	61.0	101%
1:8	30.1	29.7	99%
1:16	15.0	15.3	102%
1:32	7.5	7.3	97%
1:64	3.8	4.0	107%
1:128	1.9	2.0	108%
1:256	0.94	0.98	104%

Acetylated 2 Hour Format in 0.1 M HCl			
Dilution	**Expected (pmol/mL)**	**Observed (pmol/mL)**	**Recovery (%)**
Neat	---	27.6	---
1:2	13.8	14.7	106%
1:4	6.9	7.3	106%
1:8	3.4	3.7	106%
1:16	1.7	2.0	117%
1:32	0.86	1.1	122%
1:64	0.43	0.56	130%
1:128	0.22	0.27	127%
1:256	0.11	0.12	110%

Acetylated Overnight Format in 0.1M HCl			
Dilution	**Expected (pmol/mL)**	**Observed (pmol/mL)**	**Recovery (%)**
Neat	---	30.3	---
1:2	15.2	19.3	128%
1:4	7.6	7.4	97%
1:8	3.8	3.8	101%
1:16	1.9	2.0	106%
1:32	0.95	1.0	108%
1:64	0.47	0.53	113%
1:128	0.24	0.27	114%
1:256	0.12	0.12	98%

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of cGMP and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of cGMP in multiple assays (n=8). Different sample concentrations were used for the non-acetylated vs. acetylated format, to fall within the different standard curve ranges.

The precision numbers listed below represent the percent coefficient of variation for the concentrations of cGMP determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	Non-Acetylated Format			**Acetylated 2 Hr Format**		
	cGMP (pmol/mL)	**Intra-assay (%CV)**	**Inter-assay (%CV)**	**cGMP (pmol/mL)**	**Intra-assay (%CV)**	**Inter-assay (%CV)**
Low	1.9	4.4		0.58	9.6	
Medium	9.9	7.9		1.38	3.6	
High	115	6.6		5.38	3.5	
Low	2.1		6.0	0.35		10.9
Medium	8.5		9.9	3.51		8.4
High	97		6.9	10.3		4.6

Cross Reactivities

The cross reactivities for a number of related compounds were determined by using the cGMP ELISA kit, Catalog Number ADI-900-013, which uses the same antibody and conjugate as this kit. Potential cross reactants (purity checked by N.M.R. and other analytical methods) were dissolved in the kit Assay Buffer at a concentration of ten times the high standard, and 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%

REFERENCES

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques, 4th Ed", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
3. D.F. Ashman, et al., Biochem. Biophys. Res. Comm., (1963) 11: 330-4.
4. S.A. Waldman, and F. Murad, Pharmacol. Revs., (1987) 39: 163-197.
5. J. Tremblay, et al., Adv. 2nd Messanger & Phosphoprotein Res., (1988) 22: 319-383.
6. J. Collier, and P. Vallance, TIPS., (1989) 10: 427-431.
7. B. Sarcevic, et al., J. Biol. Chem., (1989) 264: 20648-20654.
8. M. Chinkers, et al., Nature, (1989) 338: 78-83.
9. S. Moncada, et al., Biochem. Pharmacol., (1989) 38: 1709-1715.
10. L.J. Ignarro, Pharmacol. & Toxicol., (1990) 67: 1-7
11. E.S. Bos, et al, J. Immunol., (1981) 2: 187-204.

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

Catalog Number: ADI-900-014 Rev. 021220