

## **cGMP ELISA Kit**

Catalog number: *ADI-900-013*

*96 well kit*

For the latest product information, including support documentation, visit us online:

[www.enzolifesciences.com](http://www.enzolifesciences.com)

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



# Product Manual

## **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.**

## TABLE OF CONTENTS



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



Please read entire booklet before proceeding with the assay.



Please contact Enzo Life Sciences Technical Support if necessary.

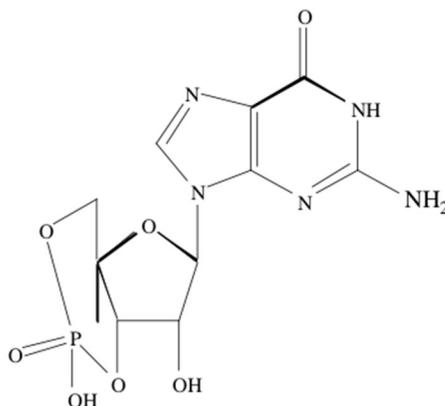
INTRODUCTION .....	2
SAFETY WARNINGS & PRECAUTIONS .....	3
MATERIALS SUPPLIED .....	3
STORAGE .....	5
ADDITIONAL MATERIALS NEEDED .....	5
SAMPLE HANDLING .....	5
PROCEDURAL NOTES .....	7
REAGENT PREPARATION .....	7
ASSAY PROCEDURE .....	9
CALCULATION OF RESULTS .....	10
TYPICAL RESULTS .....	11
TYPICAL STANDARD CURVES .....	12
PERFORMANCE CHARACTERISTICS .....	14
REFERENCES .....	17
CONTACT INFORMATION .....	18

## INTRODUCTION

The cyclic GMP Enzyme-Linked Immunosorbent Assay (ELISA) kit is a competitive immunoassay for the quantitative determination of cyclic GMP in samples. The kit uses a polyclonal antibody to cGMP to bind, in a competitive manner, the cGMP in the standard or sample or an alkaline phosphatase molecule which has cGMP covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated read on a microplate reader at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of cGMP in either standards or samples. The measured optical density is used to calculate the concentration of cGMP. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard<sup>1</sup> or Tijssen<sup>2</sup>.

Guanosine 3', 5'-cyclic monophosphate (cyclic GMP; cGMP) was identified in 1963<sup>3</sup>. 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<sup>4,5</sup>. Stimulators of guanylate cyclase such as the vasodilators nitroprusside, nitroglycerin, sodium nitrate and nitric oxide (NO) also stimulate cGMP levels<sup>6</sup>. Peptides, such as atrial natriuretic peptide (ANP) that relax smooth muscle also increase cGMP concentrations<sup>7</sup>. cGMP has been confirmed as a second messenger for ANP<sup>8</sup>. NO can be synthesized from L-arginine and diffuse through cell membranes<sup>9,10</sup>. The interaction of NO with guanylate cyclase allows cGMP to act as a third messenger in some cells<sup>11</sup>.

**Cyclic GMP**



## SAFETY WARNINGS & PRECAUTIONS

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

1. 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.
2. Some solutions supplied in this kit are caustic; care should be taken with their use.
3. 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.
4. We tested 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.
5. The cyclic GMP Standard provided, Component No. 80-0153, is supplied in ethanolic buffer at a pH optimized to maintain cGMP integrity. Care should be taken in handling this material because of the known and unknown effects of cGMP.

**CAUTION: Some components of this Kit contain chemicals that are lachrymators, corrosive and flammable. Use with caution and wear suitable protection. Refer to the Material Safety Data Sheets available on our Web site or by fax.**

## MATERIALS SUPPLIED

1. **Goat anti-Rabbit IgG Microtiter Plate, One Plate of 96 Wells, Component number 80-0060**  
A plate using break-apart strips coated with goat antibody specific to rabbit IgG.
2. **cGMP EIA Conjugate, 5 mL, Component number 80-0151**  
A blue solution of alkaline phosphatase conjugated with cGMP.
3. **cGMP EIA Antibody, 5 mL, Component number 80-0152**  
A yellow solution of a rabbit polyclonal antibody to cGMP.
4. **Assay Buffer 2, 27 mL, Component number 80-0055**  
A buffer containing proteins and sodium azide as preservative.

5. **Wash Buffer Concentrate, 27 mL, Component number 80-1286**  
Tris buffered saline containing detergents.
6. **Cyclic GMP Standard, 0.5 mL, Component number 80-0153**  
A solution of 5,000 pmol/mL cGMP.
7. **p-Npp Substrate, 20 mL, Component number 80-0075**  
A solution of p-nitrophenyl phosphate in buffer. Ready to use.
8. **Stop Solution, 5 mL, Component number 80-0247**  
A solution of trisodium phosphate in water. Keep tightly capped. Caution: **Caustic**.
9. **Acetylation Kit, 2 vials, Component number 950-001**
  - a. **Triethylamine, 2 mL, Component number 80-0063.**  
**CAUTION: Lachrymator, Harmful Vapor, Flammable.**
  - b. **Acetic Anhydride, 1 mL, Component number 80-0064.**  
**CAUTION: Lachrymator, Corrosive, Flammable.**
10. **cGMP Assay Layout Sheet, 1 each, Component number 30-0030**
11. **Plate Sealer, 1 each, Component number 30-0012**



Storage temp

## 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.

## ADDITIONAL MATERIALS NEEDED

- Deionized or distilled water
- Precision pipets for volumes between 5 µL and 1,000 µL
- Repeater pipets for dispensing 50 µL and 200 µL
- Disposable beakers for diluting buffer concentrates
- Graduated cylinders
- A microplate shaker
- Adsorbent paper for blotting
- Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm

## SAMPLE HANDLING

The cGMP ELISA kit is compatible with cGMP samples in a wide range of matrices. Samples diluted sufficiently into Assay Buffer 2 (>1:10) can be read directly from the standard curve. Please refer to the Sample Recovery recommendations (section titled "Sample Recoveries") for details of suggested dilutions. However, the end user **must verify** that the recommended dilutions are appropriate for their samples. **Samples containing rabbit IgG may interfere with the assay.** If samples with very low levels of cGMP are to be measured we have provided reagents to acetylate samples and standards. Please refer to References 12-18 for further methods of extraction of cGMP from samples.

Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer 2. There will be a small change in the binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of cGMP in the appropriate matrix.

Urine samples may be used in the assay directly after dilution in Assay Buffer 2. Plasma samples should be drawn in tubes containing EDTA. EDTA chelates calcium and will stop phosphodiesterase activity. The plasma collected should be assayed immediately or frozen below  $-20^{\circ}\text{C}$ . Plasma samples can be diluted with Assay Buffer 2 per recommendation in section titled "Performance Characteristics". If samples cannot be diluted, the following extraction method may be used. (Add 2 mL of 95% ethanol to 1 mL of the collected plasma. Vortex for 15 seconds and let sit at room temperature for 5 minutes. Centrifuge for 10 minutes at 600 x g at room temperature. Decant the supernatant into a clean tube. These samples should be dried down, reconstituted in Assay Buffer 2 and then used directly in the assay.)

Tissue samples should be rapidly frozen in liquid nitrogen. Grind the frozen tissue to a fine powder under liquid nitrogen in a stainless steel mortar. Weigh the frozen tissue and homogenize in 10 volumes of cold 5% TCA in a glass-Teflon tissue grinder. Centrifuge at 600 x g for 10 minutes. Extract the supernatants with 3 volumes of water-saturated ether. Dry the aqueous extracts and run the reconstituted samples directly in the assay.

For cells adhering to glass or plastic, use our Direct cGMP kit, Catalog Numbers ADI-900-014 or ADI-901-014.

## PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or polypropylene tubes; avoid polystyrene.
4. Pre-rinse the pipet tip and use fresh tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. 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.
8. 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.
9. **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.**

## REAGENT PREPARATION

### 1. cGMP Standard - Non-Acetylated Version

Allow the 5,000 pmol/mL cGMP standard solution to warm to room temperature. Label six 12 x 75 mm glass tubes #1 through #6. Pipet 900 µL standard diluent (Assay Buffer 2 or Tissue Culture Media) into tube #1 and 800 µL of standard diluent into tubes #2-6. Add 100 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 #6. **The concentration of cGMP in tubes #1 through #6 will be 500, 100, 20, 4, 0.8 and 0.16 pmol/mL respectively. See cGMP Assay Layout Sheet for dilution details. Diluted standards should be used within 60 minutes of preparation.**

**2. Acetylation Reagent**

Prepare the Acetylating Reagent by adding 0.5 mL of acetic anhydride to 1 mL triethylamine. **Use the prepared reagent within 60 minutes of preparation.**

**3. cGMP Standard - Acetylated Version**

Allow the 5,000 pmol/mL cGMP standard solution to warm to room temperature. Label five 12 x 75 mm glass tubes #1 through #5. Pipet 980  $\mu$ L standard diluent (Assay Buffer 2 or Tissue Culture Media) into tube #1 and 800  $\mu$ L standard diluent into tubes #2-5. Add 20  $\mu$ L of the 5,000 pmol/mL standard to tube #1. Vortex thoroughly. Add 200  $\mu$ L of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #5.

**The concentration of cGMP in tubes #1 through #5 will be 100, 20, 4, 0.8, and 0.16 pmol/mL respectively. See cGMP Assay Layout Sheet for dilution details.**

**Acetylate all standards and samples by adding 10  $\mu$ L of the Acetylating Reagent for each 200  $\mu$ L of standard or sample. Add the reagent directly to the samples and vortex for 2 seconds.**

Label one 12 x 17 mm glass tube as the Zero Standard/NSB tube. Pipet 1 mL standard diluent into this tube. Add 50  $\mu$ L of the Acetylating Reagent to the Zero Standard/NSB tube and use in Step 2 and Step 5 in the Assay Procedure.

**Failure to acetylate the NSB and Zero standard will result in inaccurate B/Bo values. Use the acetylated standards or samples within 30 minutes.**

**4. cGMP Conjugate**

Allow the conjugate to warm to room temperature before use. Any conjugate remaining after the first usage should be divided into discreet aliquots and re-frozen at  $-20^{\circ}\text{C}$ . Avoid multiple freeze-thaws.

**5. 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, or for 3 months, whichever is earlier.

## 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.

If Acetylated Version of the kit is to be run, acetylate all standards and samples by adding 10  $\mu\text{L}$  of the Acetylating Reagent for each 200  $\mu\text{L}$  of standard or sample. Add 50  $\mu\text{L}$  of the Acetylating Reagent to the Zero Standard/NSB tube (Refer to Steps 3 and 4 in Reagent Preparation section) and use in Steps 2 and 5 below; failure to acetylate the NSB and Zero standard will result in inaccurate B/Bo values. Add the reagent directly to the samples and immediately vortex for 2 seconds. Use the acetylated standards or samples within 30 minutes.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and re-seal the bag. Store unused wells at 4°C.
2. Pipet 100  $\mu\text{L}$  of standard diluent (Assay Buffer 2 or Tissue Culture Media) into the NSB and the Bo (0 pmol/mL Standard) wells.
3. Pipet 100  $\mu\text{L}$  of Standards into the appropriate wells.
4. Pipet 100  $\mu\text{L}$  of the Samples into the appropriate wells.
5. Pipet 50  $\mu\text{L}$  of Standard Diluent into the NSB wells.
6. Pipet 50  $\mu\text{L}$  of blue Conjugate into each well except the TA and Blank wells.
7. Pipet 50  $\mu\text{L}$  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.

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 Acetylated overnight format, incubate for 18-24 hours at 4°C.**
9. Empty the contents of the wells and wash by adding 400  $\mu\text{L}$  of wash solution to every well. Repeat the wash 2 more times for a total of **3 washes**.

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. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the blank wells from all readings.

## CALCULATION OF RESULTS

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:

$$\text{Average Net OD} = \text{Average Bound OD} - \text{Average NSB OD}$$

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

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

3. Plot Percent Bound ( $B/B_o$ ) versus Concentration of cGMP for the standards. The concentration of cGMP in the unknowns can be determined by interpolation from a 4 parameter logistic curve fit.

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

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

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

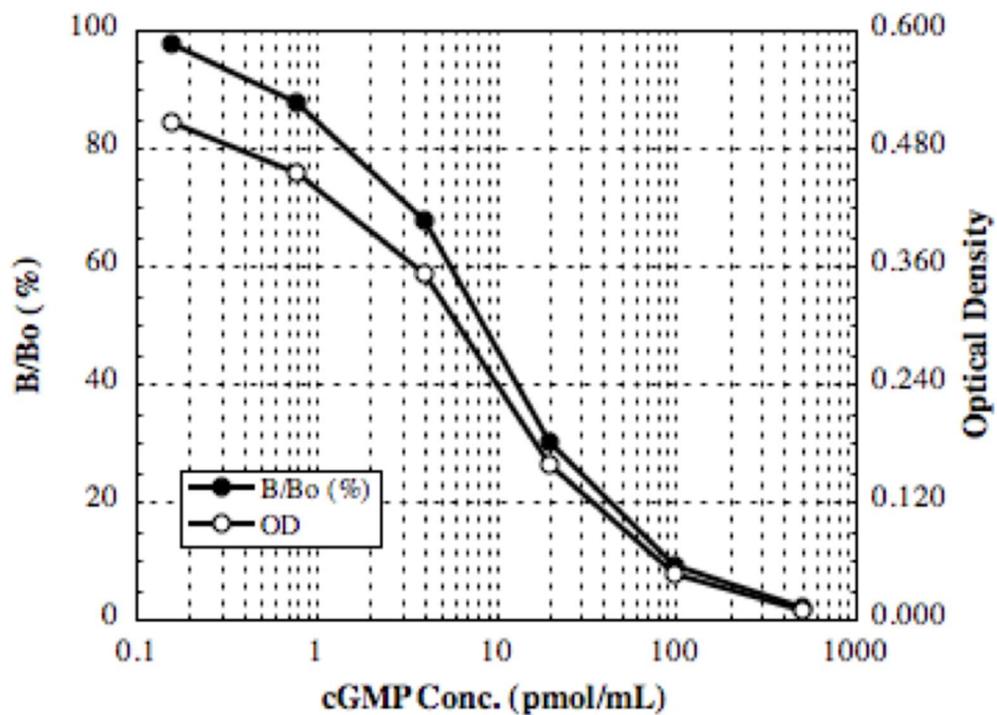
The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Non-Acetylated Version			Acetylated Version		
	Average Net OD	Percent Bound	cGMP (pmol/mL)	Average Net OD	Percent Bound	cGMP (pmol/mL)
Blank	(0.176)			(0.146)		
TA	0.296			0.320		
NSB	0.000	0%		0.002	0%	
Bo	0.519	100%	<b>0</b>	0.266	100%	<b>0</b>
S1	0.010	1.9%	<b>500</b>	0.003	1.1%	<b>100</b>
S2	0.047	9.1%	<b>100</b>	0.018	6.8%	<b>20</b>
S3	0.156	30.1%	<b>20</b>	0.062	23.3%	<b>4</b>
S4	0.352	67.8%	<b>4</b>	0.149	56.0%	<b>0.8</b>
S5	0.455	87.7%	<b>0.8</b>	0.223	83.8%	<b>0.16</b>
S6	0.507	97.7%	<b>0.16</b>	--	--	--
Unknown 1	0.300	58.9%	<b>6.05</b>	0.130	48.9%	<b>1.12</b>
Unknown 2	0.098	18.9%	<b>39.3</b>	0.010	3.8%	<b>33.9</b>

## TYPICAL STANDARD CURVES

Typical standard curves are shown below. These curves **must not** be used to calculate cGMP concentrations; each user must run a standard curve for each assay and version used.

### Non-Acetylated Version

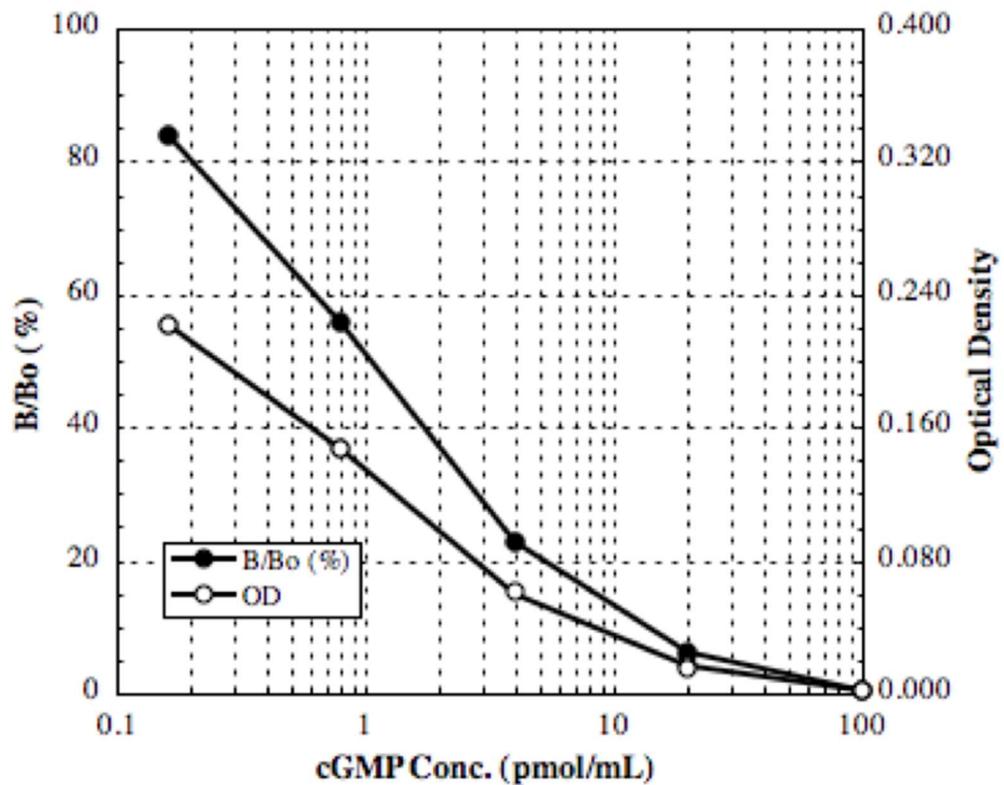


### Typical Quality Control Parameters

Quality of Fit = 0.9999  
 (Calculated from 4 parameter logistics curve fit)

20% Intercept = 36.5 pmol/mL  
 50% Intercept = 8.4 pmol/mL  
 80% Intercept = 1.9 pmol/mL

## Acetylated Version



### Typical Quality Control Parameters

Total Activity Added =  $0.320 \times 10 = 3.20$

%NSB = 0.1%

%Bo/TA = 8.3%

Quality of Fit = 1.0000

(Calculated from 4 parameter logistics curve fit)

20% Intercept = 5.1 pmol/mL

50% Intercept = 1.1 pmol/mL

80% Intercept = 0.2 pmol/mL

## PERFORMANCE CHARACTERISTICS

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols<sup>19</sup>.

### Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run with the Bo, and comparing to the average optical density for sixteen (16) wells run with Standard #6 in the Non-Acetylated, or with Standard #5 in the Acetylated version. The detection limit was determined as the concentration of cGMP measured at two (2) standard deviations from the zero along the standard curve.

#### Non-Acetylated Version

Mean OD for Bo = 0.397 ± 0.013 (3.3%)

Mean OD for Standard #6 = 0.385 ± 0.012 (3.2%)

Delta Optical Density (0-0.16 pmol/mL) = 0.397 - 0.385 = 0.012

2 SD's of the zero standard = 0.026

Sensitivity =  $\frac{0.026}{0.012} \times 0.16 \text{ pmol/mL} = \mathbf{0.37 \text{ pmol/mL}}$

#### Acetylated Version

Mean OD for Bo = 0.280 ± 0.008 (2.8%)

Mean OD for Standard #5 = 0.251 ± 0.013 (5.3%)

Delta Optical Density (0-0.16 pmol/mL) = 0.280 - 0.251 = 0.029

2SD's of the zero standard = 0.016

Sensitivity =  $\frac{0.016}{0.029} \times 0.16 \text{ pmol/mL} = \mathbf{0.088 \text{ pmol/mL}}$

## Linearity

### Non-Acetylated Version

A sample containing 800 pmol/mL cGMP was serially diluted 8 times 1:2 in the kit Assay Buffer 2 and measured in the assay. The data was plotted graphically as actual cGMP concentration versus measured cGMP concentration.

The line obtained had a slope of 1.060 with a correlation coefficient of 0.998.

### Acetylated Version

A sample containing 5.79 pmol/mL cGMP was serially diluted 3 times 1:2 in the kit Assay Buffer 2 and measured in the Acetylated version of the assay. The data was plotted graphically as actual cGMP concentration versus measured cGMP concentration.

The line obtained had a slope of 0.903 with a correlation coefficient of 0.999.

## Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of cGMP and running these samples multiple times (n=16) 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=16).

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 Version			Acetylated Version		
	cGMP (pmol/mL)	Intra-Assay (%CV)	Inter-Assay (%CV)	cGMP (pmol/mL)	Intra-Assay (%CV)	Inter-Assay (%CV)
Low	1.5	5.2		0.54	6.5	
Medium	16.6	4.0		1.50	4.6	
High	481	7.6		6.81	4.5	
Low	1.8		13.7	0.70		5.9
Medium	16.9		3.5	1.96		6.2
High	359		5.0	8.61		6.8

## Cross Reactivities

The cross reactivities for a number of related compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer 2 at concentrations from 10,000 to 10 pmol/mL. These samples were then measured in the cGMP assay, and the measured cGMP concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

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%

## Sample Recoveries

Please refer to section titled “Sample Handling” for recommendations and Standard preparation.

cGMP concentrations were measured in a variety of different samples including tissue culture media, human saliva, serum, and urine. cGMP was spiked into the undiluted samples which were diluted with the appropriate diluent and then assayed in the kit. The following results were obtained:

Sample	Non-Acetylated Version		Acetylated Version	
	% 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	1:10-1:100		Not Recommended
Human Heparin Plasma	104.4	1:10		
Human EDTA Plasma	115.0	1:10-1:100	93.6	≥ 1:2
Human Urine	97.7	1:100-1:1000		

\* See instructions in section titled “Sample Handling” for details.

**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 Messenger & 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.
12. I. Yamamoto, and J. Tsuji, Immunopharm., (1981) 3: 53-59.
13. W.P. Collins, and J.F. Hennam, In: Molecular Aspects of Medicine, (1976) H. Baum & J. Cergeley, (eds.) Pergamon, England, Vol. 1, pg 3.
14. T.W. Gettys, et al., 2nd Messengers & Phosphoprot., (1990) 13: 37.
15. T.W. Gettys, et al., J. Biol. Chem., (1991) 266: 15949.
16. A.L. Steiner, In: Meth. in Enz., (1974) J.G. Hardman & B.W. OMalley, (eds.) Academic, N.Y., Vol. 38, pg 96.
17. R.W. Farmer, et al., Anal. Biochem., (1975) 64: 455.
18. H. Fausto, and F.R. Butcher, Biochim. Biophys. Acta, (1976) 428: 702.
19. NCCLS Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.



# Product Manual

## **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**

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](http://www.enzolifesciences.com)