PerkinElmer Life and Analytical Sciences, Inc.

cGMP [^{125}I] FLASHPLATE® ASSAY (GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE) CATALOG NUMBER SMP002A

For Laboratory Use
CAUTION: A research chemical for research purposes only.

TABLE OF CONTENTS

I.	**Proprietary Name**	4
II.	**Intended Use**	4
III.	**Background Information**	4
IV	**Principle of the Procedure**	4
V.	**Reagents and Equipment**	5
VI.	**Sample Collection, Processing and Storage**	10
VII.	**Assay Procedure**	13
VIII.	**Calculations**	17
IX.	**Performance Characteristics**	18
X.	**Precautions**	22
XI.	**References**	23

I. PROPRIETARY NAME

cGMP [^{125}I] FlashPlate® Assay, Catalog Number SMP002A.

II. INTENDED USE

This product is designed to quantitatively determine cGMP (guanosine 3', 5'-cyclic monophosphate) in biological fluids.

III. BACKGROUND INFORMATION

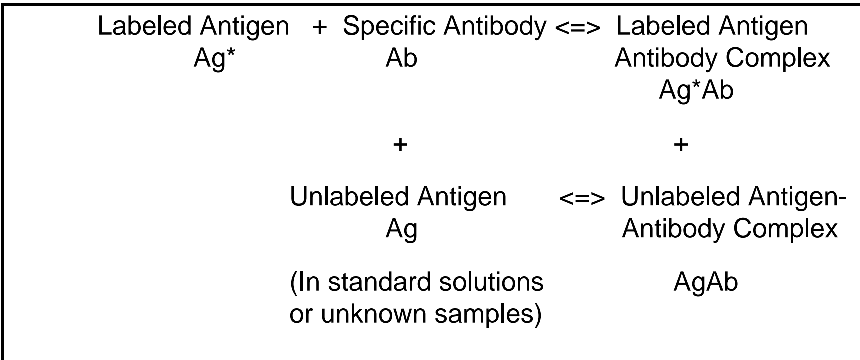
Guanosine 3', 5'-cyclic monophosphate (cyclic GMP) is a member of a family of intracellular second messenger molecules that play an important role in cell regulation. Subsequent to its discovery, it has been found that cyclic GMP (like its counterpart cyclic AMP) is widely distributed in nature occurring in all animal tissues studied as well as in prokaryotes[1]. In general, the tissue levels of cyclic GMP are 1/10 to 1/100 the concentration of cyclic AMP. This however, can vary over a wide range and, in some instances, the concentration of cyclic GMP is similar to cyclic AMP in tissues such as mouse cerebellum, rat lung and thymus gland, sperm from different sources, guinea pig macrophages, and some insects[1]. The level of cyclic GMP can be increased by stimulation of various forms of guanylate cyclase. It has been shown that nitric oxide can activate guanylate cyclase and increase accumulation of cyclic GMP to regulate smooth muscle relaxation[2,3]. More recent reviews have dealt with the role of cyclic GMP in cell function[4,5].

The [^{125}I]-cGMP FlashPlate Assay is performed using acetylated or non-acetylated standards and samples. Acetylation of the sample allows for a more sensitive assay of cGMP[6]. The assay is accurate over a wide range of values and has a high degree of specificity.

IV. PRINCIPLES OF THE PROCEDURE

The basic principle of FlashPlate Assay is the competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. This interaction is illustrated in Figure 1[7].

Figure 1



When unlabeled antigen from standards or samples and a fixed amount of the labeled antigen are allowed to react with a constant and limiting amount of antibody, decreasing amounts of the labeled antigen are bound to the antibody as the amount of unlabeled antigen is increased. In the [^{125}I]-cGMP FlashPlate Assay, the antibody is affixed to scintillant coated microplate wells and the counting of the bound fraction is dependent upon the distance of the material to the walls of the wells. Separation of the bound from the free antigen is not necessary to quantitate the bound tracer.

After incubation the antigen-antibody complex is counted and the data are used to construct a standard curve from which the values of the unknowns may be obtained by interpolation. Aspiration of the wells is recommended if the plate is going to be kept at room temperature for longer than 15 minutes. Aspiration prevents re-equilibration of the plates to the “new” ambient temperature. Also, the need for quench correcting due to color quenching by certain samples is eliminated.

V. REAGENTS AND EQUIPMENT

A. Kit Components

All necessary reagents are supplied and are intended FOR LABORATORY USE.

1. cGMP Sodium Acetate Buffer

   One vial of concentrated buffer is supplied. Dilute to 500 mL with distilled water. The final solution is in sodium acetate buffer, pH 6.2. The diluted buffer is stable for at least two months when stored at 2°-8°C.

2. cGMP Standard

One vial of lyophilized standard is supplied. Reconstitute the contents with exactly 2.0 mL of distilled water. Upon reconstitution, the resulting solution will contain 2,000 pmol/mL in sodium acetate buffer, pH 6.2. The cyclic GMP standard has been calibrated spectrophotometrically using the molar absorption coefficient for GMP, E = 13.7 x 10^3 1 mol -1 cm -1 at 252 nm, pH 7.0[8]. The reconstituted standard is stable for at least two months when stored at 2°-8°C.

3. cGMP [^{125}I] Tracer (Succinyl cGMP Tyrosine Methyl-Ester [^{125}I])

Three bottles of concentrated tracer are supplied each containing approximately 56 KBq (1.5 uCi) on calibration date in 2 mL of n-propanol:water solution (1:1). To prepare a working solution of the iodinated tracer follow the procedure described for preparation of normal rabbit serum described in section 4. The concentrate is stable for at least two months when stored at 2°-8°C. This material is radioactive and the user should follow the precautions listed on the following page.

INSTRUCTIONS RELATING TO THE HANDLING, USE, STORAGE, AND DISPOSAL OF THIS RADIOACTIVE MATERIAL

This radioactive material may be received, acquired, possessed, and used only by research laboratories for *in vitro* laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into agreement for the exercise of regulatory authority.

1. All radioactive materials must be labeled and secured in specifically designated posted areas. Records of receipt and survey must be maintained.
2. All work with these materials must be carried out only in authorized areas.
3. Prohibit mouth pipetting of radioactive materials.
4. There must be no smoking or eating within the work area.
5. Hands must be washed after handling radioactive materials.
6. Any spilled material must be wiped up quickly and thoroughly and the contaminated substances transferred to a suitable receptacle. The surfaces involved must be washed thoroughly with an appropriate decontaminant. Monitor to ensure the area has been effectively decontaminated.
7. When use of the tracer reagent has been completed, empty and decontaminate the vial. This radioactive material can be discarded into the sanitary sewerage system, using copious amounts of water to ensure a minimal discharge concentration.
8. Prior to disposal of the empty, uncontaminated kit and tracer containers to unrestricted areas, remove or deface the radioactive material labels or otherwise clearly indicate that the containers no longer contain radioactive material.

4. Normal Rabbit Serum

   Five vials of lyophilized normal rabbit serum are supplied. Use all five vials in combination with ScGMPTME [^{125}I] tracer to make working tracer

solution, enough for a 500 well assay. Reconstitute the contents of each normal rabbit serum vial with exactly 5 mL of distilled water.

To prepare the working tracer solution add 25 mL of distilled water to 5 ml (2.5 bottles) of concentrated cGMP [^{125}I] tracer. Add the entire contents of five reconstituted normal rabbit serum vials to the diluted cGMP [^{125}I] tracer. Mix well. The resulting solution, approximately 55 mL, will contain cGMP [^{125}I], normal rabbit serum, and sodium acetate buffer, pH 6.2.

The working solution is sufficiently stable to permit storage at 2°-8°C for at least two weeks. If longer storage is desired, we recommend that the freshly prepared working tracer solution be aliquoted and stored at -20°C.

5. Acetic Anhydride

One vial containing one mL is supplied. Store in the refrigerator. Allow vial to equilibrate to room temperature before use. Keep tightly closed. Protect from moisture. This material is stable for at least two months under these conditions. Refer to vial label for expiration date.

OSHA WARNING

WARNING: CORROSIVE

USA Harmful by contact. Corrosive to eyes: severely irritating to respiratory tract: affects lungs.

EU Contains: Acetic Anhydride - Flammable. Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident, or if you feel unwell, seek medical advice immediately (show the label where possible).

6. Triethylamine

One vial containing 1 mL is supplied. Store in the refrigerator. Keep tightly closed. Protect from moisture. Allow vial to equilibrate to room temperature before use. This material is stable for at least two months under these conditions. Refer to vial label for expiration date.

OSHA WARNING

> **WARNING:** IRRITANT, HIGHLY FLAMMABLE
> USA Harmful by contact, ingestion, inhalation, corrosive to eyes and skin. Severely irritating to respiratory tract. Affects central nervous system, kidneys, liver, and heart.
> EU Irritating to eyes and respiratory system. Keep away from sources of ignition—no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Do not empty into drains.

7. cGMP FlashPlate

Five microplates coated with solid scintillant to which anti-cyclic GMP antisera has been bound are supplied. Plates are shipped in an individually sealed pouch and are stable and usable for at least two months at 2°-8°C.

8. Top Seal-A Plate Cover

Ten plate covers are provided with each kit.

B. Storage of Kit Components

Kits are shipped at ambient temperature and must be stored upon receipt at refrigerator temperature (2-8°C).

C. Stability of Kit Components

The reagents are stable for the times indicated if the specific precautions given below are followed. Sodium azide has been added as an antibacterial agent where appropriate.

NOTE: The National Institute for Occupational Safety and Health has issued a bulletin citing the potentially explosive hazard due to the reaction of sodium azide with copper, lead, brass, or solder in plumbing systems. Although sodium azide is added at a minimal concentration, it is still recommended that copious amounts of water be used to flush the drain pipeline after disposal of these reagents in the plumbing system. Copper-free and lead-free discharge lines should be used whenever possible. Recommended decontamination procedures are available from Customer Technical Services.

D. Additional Equipment and Reagents Required

In addition to the reagents supplied with the kit, the following materials and equipment are required:

1. Pipettors that can accurately and precisely deliver the required volumes.
2. A 96-well microplate scintillation counter.
3. Laboratory vortex mixer.
4. Test tube rack.
5. Distilled water.
6. Test tubes - 12 x 75 mm - polypropylene.
7. 8-channel manifold, tubing, side-arm vacuum flask and vacuum pump.
8. Radioactive waste container.
9. 2 -8°C refrigerator or equivalent.

VI. SAMPLE COLLECTION, PROCESSING AND STORAGE

A. Plasma: EDTA-treated plasma should be used. Collect blood by venipuncture in a 5 or 10 mL glass blood collection tube containing EDTA, (lavender top). The usual precautions for venipuncture apply. Samples should be kept on ice after drawing. Separation of cells from plasma should be carried out as soon as possible after collection in a refrigerated centrifuge (15 minutes, approximately 760 x g). Plasma may be stored at

-20°C for at least four weeks. Repeated freezing and thawing should be avoided.

B. Urine: Voided urine specimens are required for the assay. Urine samples should be centrifuged to remove any particulate matter present. Random, timed, or 24 hour urine collections can be used. For twenty-four hour specimen, it may be necessary to prevent bacterial growth by collecting urine into acid (2 mL of 6 N HCl per 100 mL urine). Urine collected by utilizing antibacterial agents should be validated as suitable specimens by the operator. Urine samples can be stored undiluted at 2°-8°C for 24 hours, but should be frozen at -20° C for longer storage.

C. Tissue Samples: Precipitation of proteins from plasma or tissues has been accomplished with trichloroacetic acid (TCA), perchloric acid, or ethanol followed, in some cases, by ion exchange or alumina column chromatography. The decision as to which procedure to use depends on the nature of the sample and is left up to the individual investigator.

This protocol is applicable to plasma samples, tissue culture cells as well as solid tissue; however, it is the responsibility of the investigator to validate this procedure for each specific application. As part of this validation, we recommend adding phosphodiesterase to some samples and noting the loss of immunoreactivity.

1. Homogenize the frozen tissue sample at 4°C with 6% TCA to make a 1 mL 100% (w/v) homogenate. Add an equal volume of cold 10% TCA to cell culture preparations or supernatants.

2. To determine the recovery of cyclic GMP during extraction, add to each sample extract approximately 1,500 cpm of ^{3}H-cyclic GMP marker (available from PerkinElmer Cat. #NEX133T) to the TCA extract. This amount must be taken into account when calculating the cyclic GMP content of the tissue as determined by the FlashPlate Assay.

3. Centrifuge TCA extracts at 2,500 x g at 4°C for 15 minutes.

4. Collect the supernatant and extract 4 times with 5x volume of water-saturated ether. Discard the ether phase.

5. Place sample in a water bath at 70-80°C and evaporate to dryness under a stream of air.

6. Dissolve the residue in Assay Buffer. The resuspension volume depends upon the amount of cyclic GMP in the sample. Use 100 uL of sample directly in the FlashPlate Assay or dilute further with assay buffer as needed. The decision as to which procedure to use for the sample (acetylated or non acetylated) depends on expected levels of cyclic GMP in the sample.

7. If necessary, the TCA extract may be purified further by ion exchange column chromatography. In this example, it is not necessary to remove the TCA with ether as the sample may be applied directly to the column.

   a. Prepare a 0.6 x 5.0 cm column of Dowex 50w - x8(H^+), 200-400 mesh in water. This is conveniently done in a disposable capillary pipette.

   b. Prior to the sample additions to the column, it should be characterized to locate which fraction contains ^{3}H-cyclic GMP marker. Use water as the eluate.

   c. After the elution volume containing ^{3}H-cyclic GMP has been determined, allow the water to drain into the resin bed, pipette 1 mL of the TCA extract onto the column and start collecting 1 mL samples.

   d. After the TCA extract has drained into the column, add water and continue to collect the effluent.

   e. Combine the fractions previously determined to contain ^{3}H-cyclic GMP marker and continue at step 5. We have found recoveries > 90% with this procedure.

   If an excess of cyclic GMP is suspected in the sample, dilute with assay buffer.

VII. ASSAY PROCEDURE

A. Non-Acetylated Procedure

1. Standards are prepared in Assay Buffer. Mix each solution thoroughly before adding it to the next tube. Prepare the standards fresh each day.

Table I - Suggested Dilution Scheme

Pipette mL cGMP Std.	From Tube	Add mL Buffer	Into Tube	Concentration (pmol/0.1 mL)
0.2	*	3.8	A	10.0
2.0	A	2.0	B	5.0
2.0	B	2.0	C	2.5
2.0	C	3.0	D	1.0
2.0	D	2.0	E	0.50
2.0	E	2.0	F	0.25
2.0	F	3.0	G	0.10
2.0	G	2.0	H	0.05

* Reconstituted 2,000 pmol/mL (cGMP Standard Concentrate)

2. Samples should be diluted with Assay Buffer. The extent of these dilutions are sample dependent according to the amounts of cGMP expected.

NOTE: It is recommended that standards and samples be assayed in duplicate.

3. To prepare the working tracer solution add 25 mL of distilled water to 5 ml (2.5 bottles) of concentrated cGMP [^{125}I] tracer. Add the entire contents of five reconstituted normal rabbit serum vials to the diluted cGMP [^{125}I] tracer. Mix well. The resulting solution, approximately 55 mL, is enough for 500 wells.

4. Add 100 uL of Assay Buffer to wells designated for the zero standard.

5. Add 100 uL of each diluted standard to the appropriated wells.

6. A non-specific binding point (for background calculation) may be created by adding 100 uL of stock cGMP Standard Concentrate (2,000 pmol/mL) to the well followed by tracer. The cold standard competes for all of the binding sites available to ^{125}I tracer.

7. Add 100 uL of each diluted sample to the appropriate wells.

8. Add 100 uL of working tracer to all wells that have been set up for assay and also to two test tubes (12 x 75 mm) if total counts are desired.

NOTE: All participating wells should have 200 uL of liquid.

9. Place a plate cover on the plate and incubate overnight (16-24 hours) at 2°-8°C.

10. After incubation, count on a 96-well microplate scintillation counter for one minute per well.

NOTE: If FlashPlate cannot be counted within 15 minutes after removal from 2°-8°C, aspiration of the contents of the wells is recommended to prevent re-equilibration of the assay at the new temperature.

11. If all wells have not been used, return plate to refrigerated storage until ready to use.

B. Alternative Non-Acetylated Procedure

The non-Acetylated assay may be incubated at room temperature overnight. This will result in a drop in Bo and slight shift in the standard curve to a less sensitive position. The addition of a 20 and 40 pmol/0.1 mL standard is recommended. See Table IV, Figure 2 for typical assay data.

C. Acetylated Procedure

1. Standards are prepared in Assay Buffer. Mix each solution thoroughly before adding it to the next tube. Prepare the standards fresh each day.

Table II - Suggested Dilution Scheme

Pipette mL cGMP Std.	From Tube	Add mL Buffer	Into Tube	Concentration (pmol/0.1 mL)
0.1	*	1.9	Stock	10
0.2	Stock	3.8	A	0.5
2.0	A	2.0	B	0.25
2.0	B	3.0	C	0.1
2.0	C	2.0	D	0.05
2.0	D	2.0	E	0.025
2.0	E	3.0	F	0.010
2.0	F	2.0	G	0.005
2.0	G	2.0	H	0.0025

* Reconstituted 2,000 pmol/mL (cGMP Standard Concentrate)

Dilutions A through H should be used for the standard curve.

2. Samples should be diluted with Assay Buffer. The extent of these dilutions are sample dependent according to the amounts of cGMP expected.

NOTE: It is recommended that standards and samples be assayed in duplicate.

3. Acetylation of standards and samples must be done in separate test tubes.

4. Add 105 uL of each standard level and each sample to test tubes.

5. Add 105 uL of Assay Buffer to a test tube for the zero standard.

6. A non-specific binding point may be created by acetylating 105 uL of stock cGMP Standard (2,000 pmol/mL) with 5 uL of acetylating reagent. Transfer 100 uL to the well followed by tracer. The acetylated

cold standard competes for all of the binding sites available to ^{125}I tracer.

7. Acetylation: Immediately prior to use, mix one volume of acetic anhydride with two volumes of triethylamine, the exact amount is dependent on the number of samples/standards to be acetylated.

8. Add 5 uL of the above acetylating reagent to each tube. Mix well by vortexing and let incubate at room temperature for at least three minutes. More than 25,000 pmol of cGMP can be acetylated under these conditions.

9. To prepare the working tracer solution add 25 mL of distilled water to 5 ml (2.5 bottles) of concentrated cGMP [^{125}I] tracer. Add the entire contents of five reconstituted normal rabbit serum vials to the diluted cGMP [^{125}I] tracer. Mix well. The resulting solution, approximately 55 mL, is enough for 500 wells.

10. Transfer 100 uL of acetylated Assay Buffer to wells designated for the zero standard.

11. Transfer 100 uL of each acetylated standard to the appropriate wells.

12. Transfer 100 uL of each acetylated sample to the appropriate wells.

13. Add 100 uL of working tracer to all wells that have been set up for assay and also two test tubes (12 x 75 mm) if total counts are desired.

NOTE: All participating wells should have 200 uL of liquid.

14. Place a plate cover on the plate and incubate overnight (16-24 hours) at 2-8°C.

15. After incubation, count on a 96-well microplate scintillation counter for one minute per well.

NOTE: If FlashPlate cannot be counted within 15 minutes after removal from 2°-8°C, aspiration of the contents of the wells is recommended to prevent re-equilibration of the assay at the new temperature.

16. If all wells have not been used, return plate to refrigerated storage until ready to use.

D. Alternative Acetylated Procedure

The Acetylated assay may be incubated at room temperature overnight. This will result in a drop in Bo and slight shift in the standard curve to a less sensitive position. The addition of a 1.0 pmol/0.1 mL standard is recommended. See Table VI, Figure 2 for typical assay data.

VIII. CALCULATIONS

NOTE: Sample values are determined by interpolation from their respective standard curves, then corrected for appropriate dilution. The range of standard concentrations on a non-acetylated standard curve is from 0-10 pmol/0.1 mL. The range of standard concentrations on an acetylated standard curve is from 0-0.5 pmol/0.1 mL.

A. If all tubes have been counted for the same period of time, use the total accumulated counts; otherwise, correct all counts to a common count rate.

B. Average the counts for each set of duplicates, including NSB wells, if appropriate.

C. Subtract the average NSB counts from each set of duplicates to determine the average net counts for each set.

D. Express the average net counts for each standard and sample as a percentage of the average counts for the net zero standard. (This is termed “normalized” percent bound or % B/Bo).

$$\%B/Bo = \frac{\text{Average Net Counts of Standard or Sample}}{\text{Average Net Counts of Zero Standard}} \times 100$$

E. Using semi-logarithmic or log-logit graph paper or an equivalent software package, plot % B/Bo for each standard against the corresponding concentration of cGMP added in picomoles (pmol).

(See Fig. 2 for a typical standard curve using the standard protocol).

F. Determine the concentration of cGMP in each sample by interpolation from the standard curve. Since the standard curve is expressed as pmol of cGMP added, sample values must then be corrected for aliquots, dilution, recovery, etc. to determine the original concentration in the sample.

NOTE: Any samples with concentrations which are above the range of the standard curve may be diluted with Assay Buffer and re-assayed. The values obtained are then multiplied by the appropriate dilution factor.

IX. PERFORMANCE CHARACTERISTICS

A. Reproducibility

Within plate precision was determined by averaging multiple standards curves assayed on one plate each for acetylated and non-acetylated standards. Between plate precision was determined by averaging duplicate non-acetylated and acetylated standard curves from several plates.

Non-Acetylated Assay

Within Plate Variation				Between Plate Variation			
Standard pmol/mL	**n**	**Mean ± 1 S.D. cpm**	**Coeff of Var. (%)**	**Standard pmol/mL**	**n**	**Mean ± 1 S.D. cpm**	**Coeff of Var. (%)**
0	12	3949 ± 141	3.6	0	6	3900 ± 173	4.4
0.10	12	3388 ± 139	4.1	0.10	6	3473 ± 214	6.1
0.25	12	2983 ± 117	3.9	0.25	6	3072 ± 188	6.1
0.5	12	2697 ± 90	3.3	0.5	6	2716 ± 143	5.3
1.0	12	2214 ± 89	4.0	1.0	6	2279 ± 97	4.3
2.5	12	1543 ± 93	6.0	2.5	6	1576 ± 115	7.3
5	12	1068 ± 77	7.2	5	6	1116 ± 56	5.0
10	12	693 ± 42	6.1	10	6	709 ± 49	6.9

Acetylated Assay

Within Plate Variation				Between Plate Variation			
Standard pmol/mL	n	Mean ± 1 S.D. cpm	Coeff of Var. (%)	Standard Pmol/mL	n	Mean ± 1 S.D. cpm	Coeff of Var. (%)
0	12	3408 ± 139	4.1	0	6	3132 ± 162	5.2
0.005	12	3198 ± 133	4.1	0.005	6	2967 ± 195	6.6
0.01	12	2783 ± 282	10.1	0.01	6	2580 ± 231	8.9
0.025	12	2220 ± 100	4.5	0.025	6	2082 ± 116	5.6
0.05	12	1601 ± 65	4.1	0.05	6	1504 ± 83	5.5
0.1	12	1064 ± 48	4.5	0.1	6	1007 ± 58	5.7
0.25	12	543 ± 40	7.3	0.25	6	513 ± 21	4.0
0.5	12	309 ± 17	5.5	0.5	6	288 ± 10	3.6

B. Sensitivity

The assay sensitivity is defined as the concentration of cyclic GMP required to inhibit the assay by twice the standard deviation. The mean and standard deviation were determined for 7 duplicate measurments of the zero standard. Sensitivity for the non-acetylated assay was calculated as 0.06 pmol added to 0.1 mL. Sensitivity for the acetylated assay was calculated as 0.005 pmol added to 0.1 mL.

C. Specificity

The following compounds have been checked for cross-reactivity. The percentages are calculated at the 50% B/Bo point.

Compound	% Cross Reactivity	
	Non-Acetylated	Acetylated
cGmp	100	100
cAMP	.017	.04
GMP	< .0001	< .0008
GDP	< .0001	< .0008
GTP	< .0001	< .0008
ATP	< .0004	< .0008

D. Typical Results

Table III
Typical Data for Non-Acetylated Assay +4°C

Standard (pmol/0.1 mL)	CPM	Average	Norm % Bound
NSB	82/88	0	
“0” Standard	4405/4280	4258	100
0.1	3984/3874	3844	90
0.25	3565/3681	3538	83
0.5	3229/3195	3127	73
1.0	2823/2744	2699	63
2.5	1794/1743	1684	40
5.0	1216/1273	1160	27
10	854/841	763	18

Table IV
Typical Data for Non-Acetylated Assay RT

Standard (pmol/0.1 mL)	CPM	Average	Norm % Bound
NSB	123/94	0	
“0” Standard	2538/2495	2408	100
0.5	2399/2341	2262	94
1.0	2019/2055	1929	80
2.5	1685/1775	1622	67
5	1330/1378	1246	52
10	1118/1071	986	41
20	748/724	628	26
40	497/485	383	16

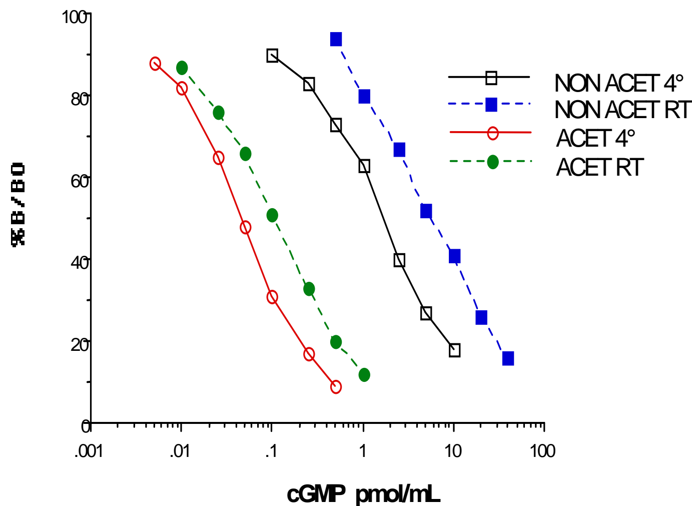
Table V
Typical Data for Acetylated Assay +4°C

Standard (pmol/0.1 mL)	CPM	Average	Norm % Bound
NSB	33/38	0	
“0” Standard	3597/3574	3550	100
0.005	3142/3187	3129	88
0.01	2807/3073	2905	82
0.025	2364/2355	2324	65
0.05	1757/1717	1702	48
0.1	1153/1110	1096	31
0.25	681/594	602	17
0.5	385/347	331	9

Table VI
Typical Data for Acetylated Assay RT

Standard (pmol/0.1 mL)	CPM	Average	Norm % Bound
NSB	81/55	0	
“0” Standard	1753/1780	1699	100
0.01	1539/1551	1477	87
0.025	1342/1372	1289	76
0.05	1208/1175	1124	66
0.1	939/932	868	51
0.25	626/624	557	33
0.5	423/382	335	20
1.0	268/260	196	12

Typical Standard Curve
Do not use to calculate samples
Figure 2
cGMP Typical Standard Curves



X. PRECAUTIONS

A. Incubation conditions should be standardized for proper day to day internal quality control.

B. As with all immunoassay procedures, pipetting is crucial. It is essential that pipetting be accurate and reproducible.

C. Samples with concentrations above the range of the standard curve may be reassayed after dilution with Assay Buffer. The values obtained are then multiplied by the appropriate dilution factor.

D. The use of grossly hemolyzed or lipemic samples should be avoided.

E. The reagents in this kit should be used as a unit. Do not mix different lots of any component within a given assay.

F. This product has not been tested for use with any methods other than those stated in this Instruction Manual.

WARNING: This product contains a chemical known to the state of California to cause cancer. (Note: ^{125}I Tracer)

XI. REFERENCES

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