StressMarq Biosciences INC.

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StressXpress®

Cyclic GMP CLIA Kit (High-Sensitivity)

Catalog# SKT-210 (96 well kit)

Chemiluminescent detection of cGMP

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-210A	Coated White 96 Well Plates	1 Each	A white plastic microtiter plate with break-apart strips coated with goat anti-mouse IgG.
SKC-210B	Cyclic GMP Standard	70 µL	Cyclic GMP at 640 pmol/mL in a special stabilizing solution.
SKC-210C	StressXpress® Cyclic GMP CLIA Antibody	3 mL	A mouse monoclonal antibody specific for cyclic GMP.
SKC-210D	StressXpress® Cyclic GMP CLIA Conjugate Stock	150 µL	A cyclic GMP-peroxidase conjugate concentrate in a special stabilizing solution.
SKC-210E	Conjugate Diluent	3 mL	Contains special stabilizers and additives.
SKC-210F	Sample Diluent	28 mL	Contains special stabilizers and additives. Ready-to-use Sample Diluent. CAUSTIC
SKC-210G	Plate Primer	5 mL	A neutralizing solution containing special stabilizers and additivies.
SKC-210H	Acetic Anhydride	2mL	WARNING: Corrosive Lachrymator
SKC-210I	Triethylamine	4mL	WARNING: Corrosive Lachrymator
SKC-210J	Wash Buffer Concentrate	30 mL	A 20X concentrate that must be diluted with deionized or distilled water.
SKC-210K	Substrate Solution A	6 mL	-
SKC-210L	Substrate Solution B	6 mL	-
SKC-210M	Plate Sealer	1 Each	-

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

- As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete booklet should be read and understood before attempting to use the product.
- The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly
- This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 10.
- The supplied Sample Diluent and Sample Diluent Concentrate are acidic. Take appropriate precautions when handling these reagents. The kit uses acetic anhydride and triethylamine as acetylation reagents. Triethylamine and acetic anhydride are lachrymators. Caution - corrosive, flammable, and harmful vapor. Use in hood with proper ventilation and wear appropriate protective safety wear.

Storage

All components of this kit should be stored at 4°C until the expiration date of the kit.

Materials Needed But Not Supplied

- Distilled or deionized water.
- Repeater pipet and tips capable of dispensing 25 and 100 µL.
- Glass test tubes.
- Microplate shaker.
- 96 well microplate reader capable of reading glow chemiluminescence. All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol:
- Dilute 5 µL of the Cyclic GMP CLIA Conjugate Concentrate into 1.495 mL of deionized water. Pipet 5 µL of diluted conjugate into 45 µL of deionized water. Pipet 5 µL of this mixture into a white well and add 100 µL of prepared CLIA substrate (see page 11 for details). This well will give you an intensity slightly above the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the maximum signal.
- To properly analyze the data software will be required for converting raw RLU readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Please read this booklet completely prior to using the product.

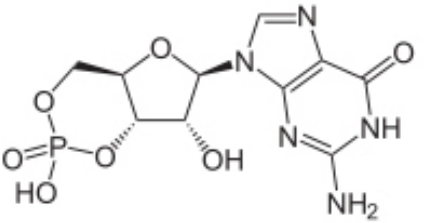
FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Guanosine 3', 5'-cyclic monophosphate (cyclic GMP; cGMP) is a critical and multifunctional second messenger present at levels typically 10-100 fold lower than cAMP in most tissues. Intracellular cGMP is formed by the action of the enzyme guanylate cyclase on GTP and degraded through phosphodiesterase hydrolysis (1-3). Guanylate cyclases (GC) are either soluble or membrane bound (3,4). Soluble GCs are nitric oxide responsive, whereas membrane bound GCs respond to hormones such as acetylcholine, insulin and oxytocin. Other chemicals like serotonin and histamine also cause an increase in cGMP levels (5,6). Cyclic GMP regulates cellular composition through cGMP-dependent kinase, cGMP-dependent ion channels or transporters, and through its hydrolytic degradation by phosphodiesterase (1,7).



Assay Overview

The Direct High Sensitivity Cyclic GMP (cGMP) *StressXpress*® CLIA kit is designed to quantitatively measure cGMP present in lysed cells, EDTA plasma, urine, saliva and tissue culture media samples. Please read the complete kit booklet before performing this assay.

For samples where the levels of cGMP are expected to be relatively high, the regular format for the assay can be used. For samples with expected low levels of cGMP, an optional acetylation protocol can be used.

The kit is unique in that all samples and standards are diluted into an acidic Sample Diluent, which contains special additives and stabilizers, for cGMP measurement. This allows plasma, urine and saliva samples to be read in an identical manner to lysed cells. Acidified samples of cGMP are stable and endogenous phosphodiesterases are inactivated in the Sample Diluent. A cGMP standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. A white microtiter plate coated with an antibody to capture mouse IgG is provided and a neutralizing Plate Primer solution is added to all the used wells. Standards or diluted samples, either with or without acetylation, are pipetted into the primed wells. A cGMP-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a mouse monoclonal antibody to cGMP to each well. After an overnight incubation at 4°C, the plate is washed and the chemiluminescent substrate is added. The substrate reacts with the bound cGMP-peroxidase conjugate to produce light The generated light is detected in a microtiter plate reader capable of reading luminescence. The concentration of the cGMP in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

Sample Types

Sample Types Validated:
Cell Lysates, Saliva, Urine, EDTA Plasma and Tissue Culture Media

This assay has been validated for lysed cells, saliva, urine, EDTA plasma samples and for tissue culture media samples. Samples should be stored at -70°C for long term storage. 24-Hour urine samples may need to have 1 mL concentrated hydrochloric acid added for every 100 mL volume to act as a preservative. Samples containing visible particulate should be centrifuged prior to using.

Cyclic GMP is identical across all species and we expect this kit may measure cGMP from sources other than human. The end user should evaluate recoveries of cGMP in other samples being tested.

After dilution in the Sample Diluent (see page 11) there may be some precipitation of proteins and the supernatant from the centrifuged samples used. After being diluted in Sample Diluent the samples can be assayed directly within 2 hours, or frozen at ≤ -70°C for later analysis. Severely hemolyzed samples should not be used in this kit.

For samples containing low levels of cGMP, the acetylated assay protocol must be used due to its enhanced sensitivity. All standards and samples should be diluted in glass test tubes.

Sample Preparation

Cells

Cell lysis buffers containing high concentrations of SDS or other detergents may not be compatible with this assay or may require extra dilution. Please read Interferents section on page 28 for more information.

This kit is compatible with either adherent or non-adherent cells. The cells can be grown in any suitable sterile containers such as Petri dishes, 12-, 48- or 96-well culture plates or flasks. The cells must be isolated from the media prior to being lysed with the provided Sample Diluent. The acidic Sample Diluent contains detergents to lyse the cells, inactivate endogenous phosphodiesterases and stabilize the cGMP. Some cell types are extremely hardy and the end user should optimize the lysis conditions utilizing freeze-thaw cycles and ultrasonic treatments to fully lyse their cells.

We used ~ 107 Jurkat cells per mL of Sample Diluent. Cell number needs to be determined by the end user since it will be dependant on cell type and treatment conditions. Care must be taken not to over dilute the samples.

For adherent cells, the media should be aspirated from the cells and the cells washed with PBS. The adherent cells should be treated directly with the Sample Diluent for 10 minutes at room temperature. Cells can be scraped to dislodge them from the plate surface and cells should be inspected to ensure lysis. Detergent has been added to the Sample Diluent to help lysis occur. Centrifuge the samples at ≥600 x g at 4°C for 15 minutes and assay the supernatant directly. If required, the TCM can be assayed for cGMP as outlined below.

For non-adherent cells, pellet and wash the cells with PBS by centrifuging the samples at ≥600 x g at 4°C for 15 minutes as described above. Treat the aspirated, washed pellet directly with the Sample Diluent for 10 minutes at room temperature. Cells should be inspected to ensure lysis. Detergent has been added to the Sample Diluent to help lysis occur. Centrifuge the samples at ≥600 x g at 4 °C for 15 minutes and assay the supernatant directly. If required, the TCM can be assayed for cGMP as outlined below.

Tissue Samples

Tissues samples should be frozen in liquid nitrogen and stored at -80°C if analysis is not to be carried out immediately.

Grind the frozen tissue in a stainless steel mortar under liquid nitrogen until it is a fine powder. Allow the liquid nitrogen to evaporate and weigh the powdered tissue. Add 1 mL of Sample Diluent for every 100 mg of tissue. Incubate in the Sample Diluent for 10 minutes on ice, and then centrifuge at ≥600 x g at 4°C for 15 minutes. Collect the supernatant and run in the assay immediately or store frozen at ≤ -70°C.

For samples that require concentration and delipidation, a trichloroacetic acid (TCA)/ether protocol can be used. Grind the frozen tissue in a stainless steel mortar under liquid nitrogen until it is a fine powder. Allow the liquid nitrogen to evaporate and weigh the powdered tissue. Add 1 mL of ice cold 5% TCA (weight/volume) for every 100 mg of tissue and grind in a glass-Teflon mortar. Incubate in the TCA for 10 minutes on ice, and then centrifuge at ≥600 x g at 4°C for 15 minutes. Collect the supernatant.

For every 1 mL of TCA supernatant add 3 mL of water saturated diethyl ether* and shake in a glass vial. Allow the ether to separate as the top layer, remove it and discard the ether. Dry the aqueous layer by lyophilization or using a vacuum centrifuge. Reconstitute by adding 1 mL of Sample Diluent for every mL of 5% TCA used to extract and run in the assay immediately or store at ≤ -70°C.
*Diethyl ether is extremely flammable and should be used in a hood.

Tissue Culture Media

For measuring cGMP in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Plasma Samples

Plasma samples should be diluted ≥ 1:10 with the supplied Sample Diluent and acetylated prior to running in the Acetylated Format assay (page 18).

Urine Samples

Urine samples should be diluted ≥ 1:20 with the supplied Sample Diluent prior running in the assay. Due to the high concentration of cGMP in urine, samples may need to be diluted further.

Saliva Samples

Saliva samples should be diluted ≥ 1:4 with the supplied Sample Diluent prior running in the assay. See our Saliva Sample Handling Instructions available on this product's website page.

Use all samples within 2 hours of dilution in Sample Diluent.

Reagent Preparation

Allow the kit reagents to thaw and come to room temperature for 30-60 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine cGMP concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Do not dilute the Sample Diluent (Catalog Number SKC-210F).

Cyclic GMP Conjugate

The supplied Cyclic GMP CLIA Conjugate Concentrate should be diluted 1:20 with the Conjugate Diluent as indicated in the table below. Once diluted the Cyclic GMP conjugate is stable for one month when stored at 4°C.

	1 Plate	2 Plates	3 Plates	4 Plates	5 Plates
Conjugate Concentrate	125 µL	250 µL	375 µL	500 µL	625 µL
Conjugate Diluent	2.375 mL	4.75 mL	7.125 mL	9.5 mL	11.875 mL
Final Mixture	2.5 mL	5 mL	7.5 mL	10 mL	12.5 mL

Chemiluminescent Substrate

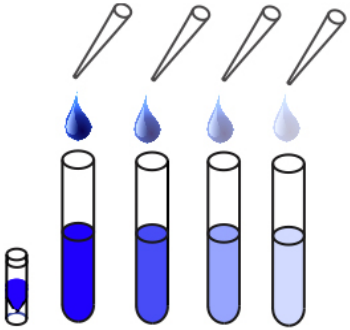
Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle. Once mixed the substrate is stable for one month when stored at 4°C.

Reagent Preparation - Regular Format

All standards and samples should be diluted in glass test tubes.

Standard Preparation - Regular Format

Label one glass test tube as Stock 2 and seven tubes as #1 through #7. Pipet 150 µL of Sample Diluent into the Stock 2 tube and 296 µL of Sample Diluent into tube #1. Pipet 150 µL of Sample Diluent into tubes #2 to #7. The Cyclic GMP stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 10 µL of the cGMP stock solution to the Stock 2 tube and vortex completely. Take 24 µL of the cGMP solution in the Stock 2 tube and add it to tube #1 and vortex completely. Take 150 µL of the cGMP solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of Cyclic GMP in tubes 1 through 7 will be 3, 1.5, 0.75, 0.375, 0.188, 0.094, and 0.047pmol/mL.



Non-Acetylated	Stock 2	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Sample Diluent (µL)	150	296	150	150	150	150	150	150
Addition	Cyclic GMP Standard	Stock 2	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Volume of Addition (µL)	10	24	150	150	150	150	150	150
Final Concentration (pM/mL)	40	3	1.5	0.75	0.375	0.1875	0.0938	0.0469

Use Standards within 1 hour of preparation.

Acetylated Protocol - Overview

Use this format for any sample with low cGMP concentrations.

Prior to running the acetylated assay, all standards, samples and the Sample Diluent used for the B0 and NSB wells must be acetylated. Acetylation is carried out by adding 10 µL of the Acetylation Reagent (as prepared below) for each 200 µL of the standard, sample and Sample Diluent. Immediately vortex each treated standard, sample or Sample Diluent after addition of the Acetylation Reagent and use within 30 minutes of preparation.

Note: Upon Acetylation, all of the standards and samples diluted in the orange Sample Diluent will change to a pale yellow colour.

Reagent Preparation - Acetylated Format

Acetylation Reagent

Working in a fume hood mix one part of Acetic Anhydride with 2 parts of Triethylamine in a glass test tube. Use the following table to help determine the amount of Acetylation Reagent to make.

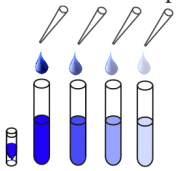
Reagents	Number of Samples to be Tested			
	20	40	100	200
Acetic Anhydride Volume (µL)	200	400	1,000	2,000
Triethylamine Volume (µL)	400	800	2,000	4,000
Acetylation Reagent Volume (mL)	0.6	1.2	3	6

Use the Acetylation Reagent within 60 minutes of preparation.

Standard Preparation - Acetylated Format

All standards and samples should be diluted in glass test tubes.

Label one glass test tube as Stock 2 and six tubes as #1 through #6. Pipet 150 µL of Sample Diluent into the Stock 2 tube and 585 µL of Sample Diluent into tube #1. Pipet 300 µL of Sample Diluent into tubes #2 to #6. The Cyclic GMP stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 10 µL of the cGMP stock solution to the Stock 2 tube and vortex completely. Take 15 µL of the cGMP solution in the Stock 2 tube and add it to tube #1 and vortex completely. Take 300 µL of the cGMP solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of Cyclic GMP in tubes 1 through 6 will be 1, 0.5, 0.25, 0.125, 0.0625, and 0.0313 pmol/mL.



Non-Acetylated	Stock 2	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Sample Diluent (µL)	150	585	300	300	300	300	300
Addition	Cyclic GMP Standard.	Stock 2	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Volume of Addition (µL)	10	15	300	300	300	300	300
Final Concentration (pM/mL)	40	1	0.5	0.25	0.125	0.0625	0.03125

Standard and Sample Acetylation

Pipet 300 µL of Sample Diluent into a glass tube to act as the Zero standard/ NSB tube. Add 15 µL of Acetylation Reagent to this tube and vortex immediately. Proceed to assay within 30 minutes.

Pipet 200 µL of each standard or sample to be tested into fresh glass tubes. Add 10 µL of the Acetylation Reagent into each tube and vortex immediately. Proceed to assay within 30 minutes.

NOTE: Samples and Sample Diluent will turn from orange to pale yellow upon acetylation.

Use Acetylated Standards and Samples within 30 minutes of preparation.

Assay Protocol - Regular Format

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Add 50 µL of Plate Primer into all wells used. Failure To Add Plate Primer To ALL Wells First Will Cause Assay To Fail.

3. Pipet 75 µL Sample Diluent into the non-specific binding (NSB) wells.

4. Pipet 50 µL of Sample Diluent into wells to act as maximum binding wells (B0 or 0 pmol/mL).

5. Pipet 50 µL of samples or standards into wells in the plate.
NOTE: Sample Diluent will turn from orange to bright pink upon sample or standard addition to the Plate Primer in the wells.

6. Add 25 µL of the diluted StressXpress® cGMP CLIA Conjugate to each well using a repeater pipet.

7. Add 25 µL of the StressXpress® cGMP CLIA Antibody to each well, except the NSB wells, using a repeater pipet.

8. Cover the plate with the plate sealer and shake the plate for 15 minutes at room temperature.

9. Place the covered plate in a 4°C refrigerator for 16 hours.

10. The next morning take the plate from the refrigerator and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

11. Add 100 µL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet.

12. Incubate the plate at room temperature for 5 minutes without shaking.

13. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40% over 60 minutes.

14. Use the plate reader's built-in 4PLC software capabilities to calculate cGMP concentration for each sample.

Assay Protocol - Acetylated Format

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Add 50 µL of Plate Primer into all wells used. Failure To Add Plate Primer To ALL Wells First Will Cause Assay To Fail.

3. Pipet 75 µL acetylated Sample Diluent into the non-specific binding (NSB) wells.

4. Pipet 50 µL of acetylated Sample Diluent into wells to act as maximum binding wells (B0 or 0 pmol/mL).

5. Pipet 50 µL of acetylated samples or standards into wells in the plate.

6. Add 25 µL of the diluted StressXpress® cGMP CLIA Conjugate to each well using a repeater pipet.

7. Add 25 µL of the StressXpress® cGMP CLIA Antibody to each well, except the NSB wells, using a repeater pipet.

8. Cover the plate with the plate sealer and shake the plate for 15 minutes at room temperature.

9. Place the covered plate in a 4°C refrigerator for 16 hours.

10. The next morning take the plate from the refrigerator and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

11. Add 100 µL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet.

12. Incubate the plate at room temperature for 5 minutes without shaking.

13. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40% over 60 minutes.

14. Use the plate reader's built-in 4PLC software capabilities to calculate cGMP concentration for each sample.

Calculation of Results

All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. Average the duplicate RLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean RLU's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

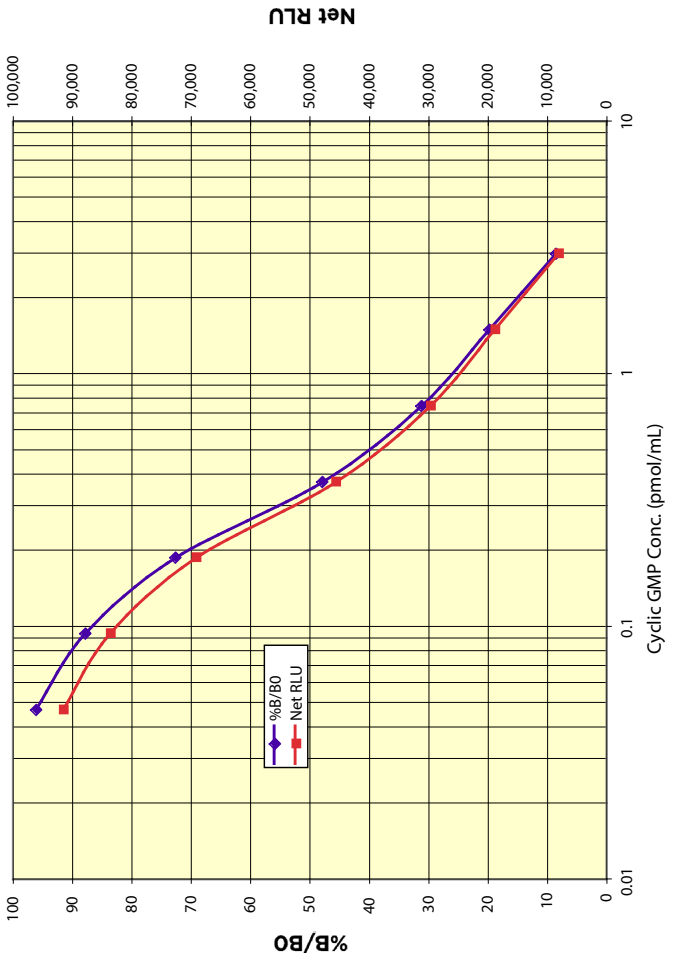
Typical Data - Regular Format

Sample	Mean RLU	Net RLU	% B/B0	Cyclic GMP Concentration (pmol/mL)
NSB	10,885	0	-	-
Standard 1	18,940	8,055	8.5	3
Standard 2	29,635	18,750	19.7	1.5
Standard 3	40,520	29,635	31.1	0.75
Standard 4	56,450	45,565	47.8	0.375
Standard 5	79,995	69,110	72.6	0.1875
Standard 6	94,450	83,565	87.7	0.0938
Standard 7	102,345	91,460	96.0	0.0469
B0	106,140	95,255	100.0	0
Sample 1	29,320	18,435	19.4	1.32
Sample 2	54,605	43,720	45.9	0.43

Always run your own standard curve for calculation of results.

Do not use this data

Typical Standard Curve - Regular Format



Validation Data - Regular Format

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and standard #7. The sensitivity was determined at two (2) standard deviations from the B0 along the standard curve.
Sensitivity was determined as 0.034 pmol/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty runs for each of the zero standard and a low concentration human urine sample.
Limit of Detection was determined as 0.047 pmol/mL

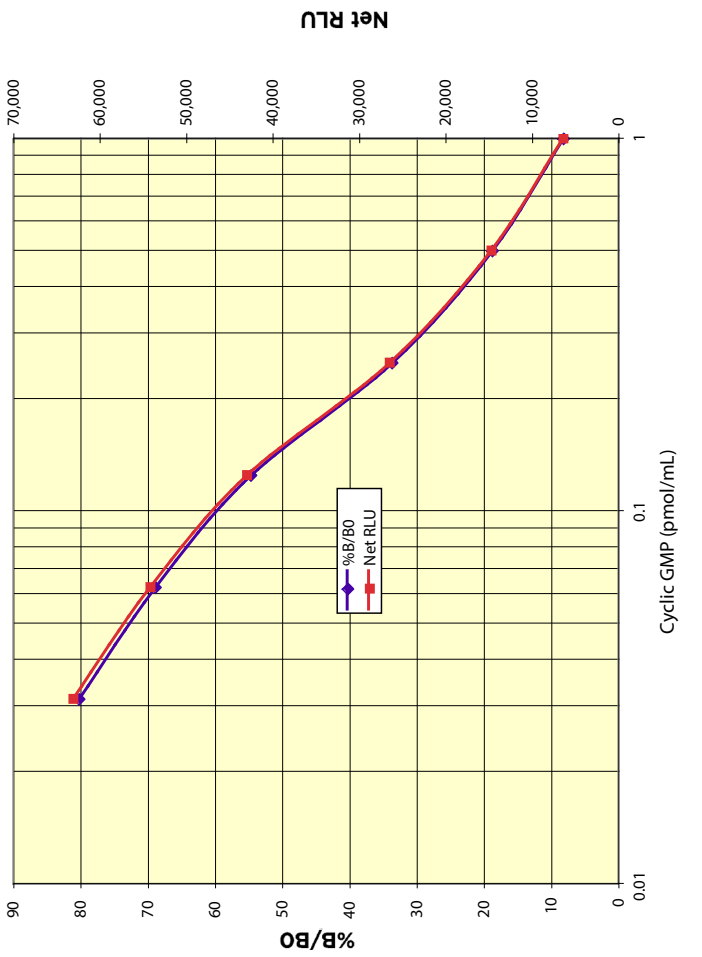
Typical Data - Acetylated

Sample	Mean RLU	Net RLU	% B/B0	Cyclic GMP Concentration (pmol/mL)
NSB	23,295	0	-	-
Standard 1	29,680	6,385	12.5	1
Standard 2	38,010	14,715	19.1	0.5
Standard 3	49,775	26,480	29.6	0.25
Standard 4	66,280	42,985	47.7	0.125
Standard 5	77,475	54,180	64.8	0.0625
Standard 6	86,390	63,095	78.3	0.0313
B0	102,000	78,705	100.0	0
Sample 1	39,960	16,665	37.4	0.433
Sample 2	69,335	46,040	65.2	0.104

Always run your own standard curve for calculation of results.

Do not use this data.

Typical Standard Curve - Acetylated



Validation Data - Acetylated Format

Sensitivity and Limit of Detection - Acetylated

Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the acetylated B0 and standard #6. The sensitivity was determined at two (2) standard deviations from the B0 along the standard curve.
Sensitivity was determined as 0.023 pmol/mL. This is equivalent to 1.15 fmol/well.

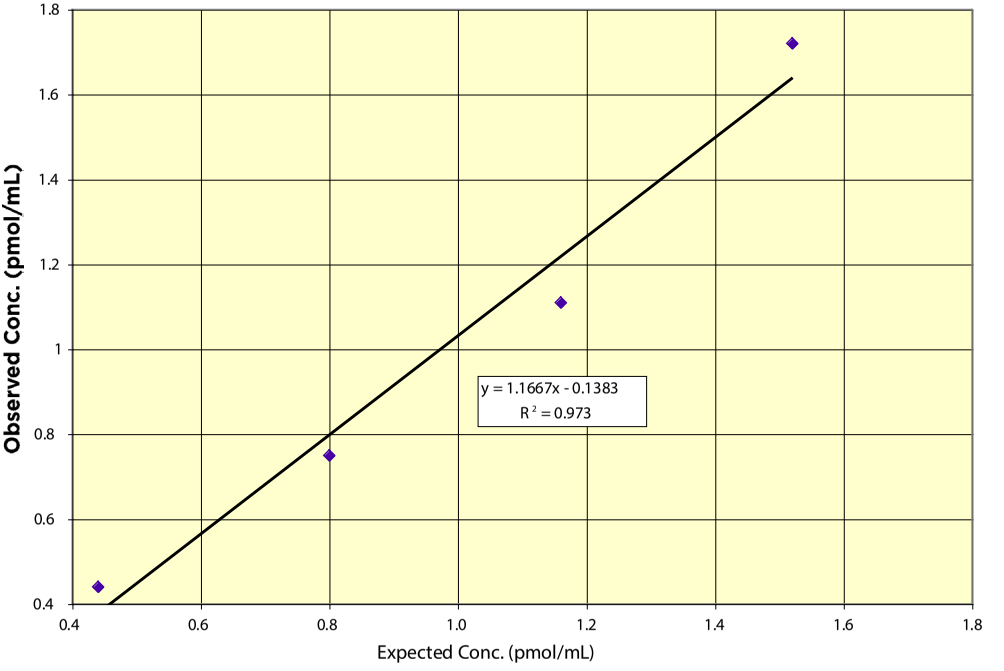
The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty runs for each of acetylated zero standard and a low concentration acetylated human sample.
Limit of Detection was determined as 0.019 pmol/mL. This is equivalent to 0.95 fmol/well.

Validation Data - Regular and Acetylated

Linearity

Linearity was determined by taking two human urine samples, one with a low cGMP level of 0.3 pmol/mL and one with a higher level of 9.7 pmol/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine	Low Urine	Observed Concentration (pmol/mL)	Expected Concentration (pmol/mL)	% Recovery
80%	20%	1.72	1.52	113.2
60%	40%	1.11	1.16	95.7
40%	60%	0.75	0.80	93.8
20%	80%	0.44	0.44	100.0
			Mean Recovery	100.3%


Observed Conc. (pmol/mL)
Expected Conc. (pmol/mL)
y = 1.1667x - 0.1383
R² = 0.973
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8

Intra Assay Precision - Regular

Two human urine samples were diluted with Sample Diluent and run in replicates of 22 in an assay. The mean and precision of the calculated cGMP concentrations were:

Sample	Cyclic GMP Concentration (pmol/mL)	%CV
1	0.053	10.6
2	0.225	11.8

Inter Assay Precision - Regular

Two human urine samples were diluted with Sample Diluent and run in duplicates in fourteen assays run over multiple days by four operators. The mean and precision of the calculated cGMP concentrations were:

Sample	Cyclic GMP Concentration (pmol/mL)	%CV
1	0.213	14.9
2	0.083	18.0

Intra Assay Precision - Acetylated

Two human urine samples were diluted with Sample Diluent, acetylated and run in replicates of twenty-two in an assay. The mean and precision of the calculated cGMP concentrations were:

Sample	Cyclic GMP Concentration (pmol/mL)	%CV
1	0.032	8.9
2	0.018	8.3

Inter Assay Precision - Acetylated

Two human urine sample weres diluted with Sample Diluent, acetylated and run in duplicates in eight assays run over multiple days by four operators. The mean and precision of the calculated cGMP concentrations were:

Sample	Cyclic GMP Concentration (pmol/mL)	%CV
1	0.07	15.1
2	0.04	15.1

Sample Values

Four human plasma samples were tested in the assay. Samples were diluted 10-20 fold and run in the assay. Values ranged from 3.0 to 8.0 pmol/mL with an average for the samples of 5.48 pmol/mL. Five normal human urine samples were diluted between 50 and 2,000 fold in Sample Diluent and values ranged in the neat samples from 44.2 to 564 pmol/mL with an average for the samples of 287.2 pmol/mL.

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Nucleotide	Cross Reactivity (%)
Cyclic GMP	100%
Cyclic AMP	< 0.1%
GMP	< 0.1%
AMP	< 0.1%
ATP	< 0.1%

Interferents

A variety of detergents were tested as possible interfering substances in the assay. CHAPS at 0.1% increased measured cGMP by 8.6% and Tween 20 at 1.0% increased measured cGMP by 6%. Triton X-100 at 2% decreased measured cGMP by 6.1%. SDS at 0.05% Samples containing decreased measured cGMP by 9%. CTAC above 0.05% should not be used in the assay.

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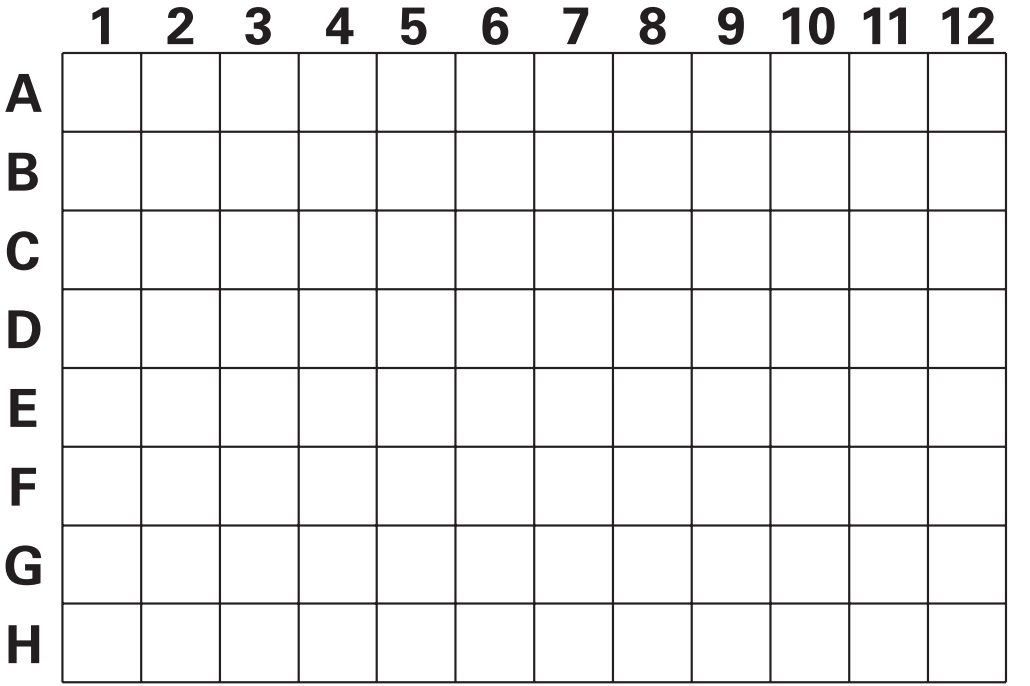
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