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StressXpress®

Cyclic GMP EIA Kit

Catalog# SKT-211 (96 well kit)

Quantitative colorimetric detection of cGMP

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GENERAL INFORMATION

Materials Supplied

| Catalog Number | Reagent | Quantity | Description |
|----------------|------------------------------------|------------|--|
| SKC-211A | Coated Clear 96 Well Plates | 1 Plate | A clear plastic microtiter plate coated with goat anti-mouse IgG. |
| SKC-211B | Cyclic GMP Standard | 70 μ L | Cyclic GMP at 640 pmol/mL in a special stabilizing solution. |
| SKC-211C | StressXpress® Cyclic GMP Antibody | 3 mL | A mouse monoclonal antibody specific for cyclic GMP. |
| SKC-211D | StressXpress® Cyclic GMP Conjugate | 3 mL | A cyclic GMP-peroxidase conjugate in a special stabilizing solution. |
| SKC-211E | Sample Diluent | 28 mL | Contains special stabilizers and additives. Ready-to-use Sample Diluent. CAUSTIC |
| SKC-211F | Plate Primer | 5 mL | A neutralizing solution containing special stabilizers and additives. |
| SKC-211G | Acetic Anhydride | 2 mL | WARNING: Corrosive Lachrymator |
| SKC-211H | Triethylamine | 4 mL | WARNING: Corrosive Lachrymator |
| SKC-211I | Wash Buffer Concentrate | 30mL | A 20X concentrate that must be diluted with deionized or distilled water. |
| SKC-211J | TMB Substrate | 11mL | - |
| SKC-211K | Stop Solution | 5 mL | - |
| SKC-211L | 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 11.

The supplied Sample Diluent and Sample Diluent Concentrate are acidic. The Stop Solution is 1M HCl. These solutions should not come in contact with skin or eyes. 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.
- Borosilicate glass test tubes.
- Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25, 50 and 100 μL .
- A microplate shaker.
- Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.
- Software for converting raw relative optical density 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 use the product.

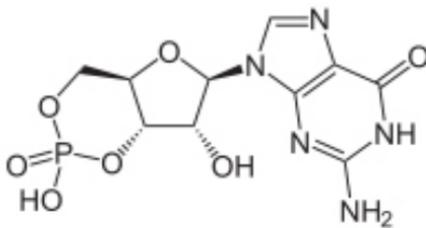
FOR RESEARCH USE ONLY.

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 Cyclic GMP (cGMP) *StressXpress*® EIA 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 clear 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 a 2 hour incubation, the plate is washed and substrate is added. The substrate reacts with the bound cGMP-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. 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 $\leq -70^{\circ}\text{C}$ 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 27 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.

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 $\geq 600 \times g$ 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 $\geq 600 \times g$ 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 $\geq 600 \times g$ 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 $\geq 600 \times g$ at 4°C for 15 minutes. Collect the supernatant and run in the assay immediately or store frozen at $\leq -70^{\circ}\text{C}$.

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 $\geq 600 \times g$ 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 $\leq -70^{\circ}\text{C}$.

*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 $\geq 1:10$ with the supplied Sample Diluent and acetylated prior to running in the Acetylated Format assay (page 17).

Urine Samples

Urine samples should be diluted $\geq 1:5$ 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 $\geq 1:4$ 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.

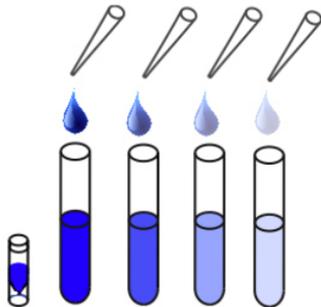
Do not dilute the Sample Diluent (Catalog Number SKC-211E).

Reagent Preparation - Regular Format

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

Standard Preparation - Regular Format

Label seven test tubes as #1 through #7. Pipet 380 μL of Sample Diluent into tube #1 and 200 μL 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 20 μL of the cGMP stock solution to tube #1 and vortex completely. Take 200 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 32, 16, 8, 4, 2, 1, and 0.5 pmol/mL. Use Standards within 1 hour of preparation.



| Non-Acetylated | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 |
|--------------------------------------|------------|------------|------------|------------|------------|------------|------------|
| Sample Diluent (μL) | 380 | 200 | 200 | 200 | 200 | 200 | 200 |
| Addition | Stock | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 |
| Volume of Addition (μL) | 20 | 200 | 200 | 200 | 200 | 200 | 200 |
| Final Concentration (pmol/mL) | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 |

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.

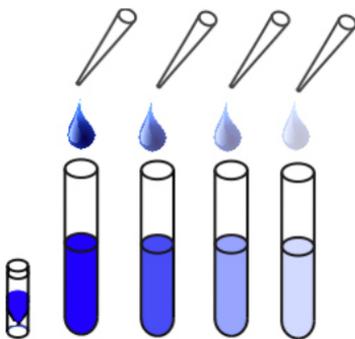
| Reagents | Number of Samples to be Tested | | | |
|---|--------------------------------|-----|-------|-------|
| | 20 | 40 | 100 | 200 |
| Acetic Anhydride Volume (μL) | 200 | 400 | 1,000 | 2,000 |
| Triethylamine Volume (μL) | 400 | 800 | 2,000 | 4,000 |
| Acetylation Reagent Volume (mL) | 0.6 | 1.2 | 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 seven test tubes as #1 through #7. Pipet 620 μL of Sample Diluent into tube #1 and 300 μL 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 20 μL of the cGMP stock solution 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 #7. The concentration of Cyclic GMP in tubes 1 through 7 will be 20, 10, 5, 2.5, 1.25, 0.625, and 0.3125 pmol/mL.



| | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 | Standard 7 |
|--------------------------------------|------------|------------|------------|------------|------------|------------|------------|
| Sample Diluent (μL) | 620 | 300 | 300 | 300 | 300 | 300 | 300 |
| Addition | Stock | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 |
| Volume of Addition (μL) | 20 | 300 | 300 | 300 | 300 | 300 | 300 |
| Final Concentration (pmol/mL) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.313 |

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

Assay Protocol - Regular Format

1. Use the plate layout sheet on page 30 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 StressXpress® cGMP Conjugate to each well using a repeater pipet.
7. Add 25 μL of the StressXpress® cGMP Antibody to each well, except the NSB wells, using a repeater pipet.
8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken, signals bound will be approximately 25% lower.
9. Aspirate the plate and wash each well 4 times with 300 μL wash buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 μL of the TMB Substrate to each well, using a repeater pipet.

11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 μL of the Stop Solution to each well, using a repeater pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
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 page 30 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 StressXpress® cGMP Conjugate to each well using a repeater pipet.
7. Add 25 μL of the StressXpress® cGMP Antibody to each well, except the NSB wells, using a repeater pipet.

8. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken, signals bound will be approximately 25% lower. NOTE: Wells will have turned from very pale yellow to pale pink during incubation.
9. Aspirate the plate and wash each well 4 times with 300 μL wash buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 μL of the TMB Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 μL of the Stop Solution to each well, using a repeater pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
14. Use the plate reader's built-in 4PLC software capabilities to calculate cGMP concentration for each sample.

ANALYSIS

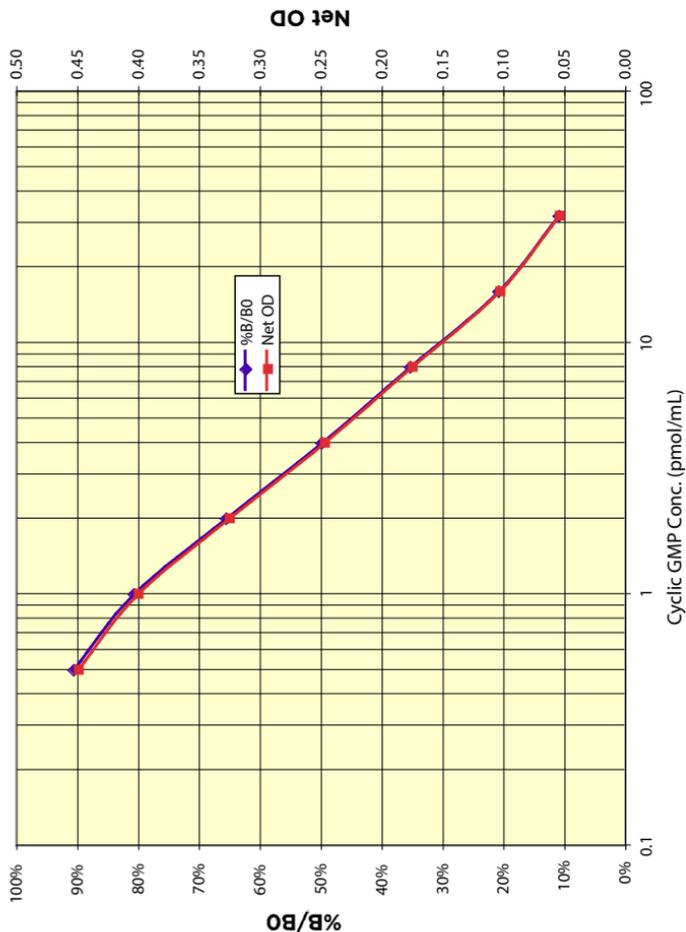
Calculation of Results

Average the duplicate OD 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 OD'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 OD | Net OD | % B/B0 | Cyclic GMP Concentration (pmol/mL) |
|------------|---------|--------|--------|------------------------------------|
| NSB | 0.047 | 0 | - | - |
| Standard 1 | 0.101 | 0.054 | 10.9 | 32 |
| Standard 2 | 0.150 | 0.103 | 20.8 | 16 |
| Standard 3 | 0.222 | 0.175 | 35.3 | 8 |
| Standard 4 | 0.294 | 0.247 | 49.8 | 4 |
| Standard 5 | 0.372 | 0.325 | 65.5 | 2 |
| Standard 6 | 0.447 | 0.400 | 80.6 | 1 |
| Standard 7 | 0.496 | 0.449 | 90.5 | 0.5 |
| B0 | 0.543 | 0.496 | 100.0 | 0 |
| Sample 1 | 0.207 | 0.160 | 32.3 | 8.93 |
| Sample 2 | 0.422 | 0.375 | 75.6 | 1.26 |

Typical Standard Curve - Regular Format



Always run your own standard curve for calculation of results.
Do not use this data.

Validation Data - Regular Format

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD'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 0.28 pmol/mL.

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

Limit of Detection was determined as 0.26 pmol/mL

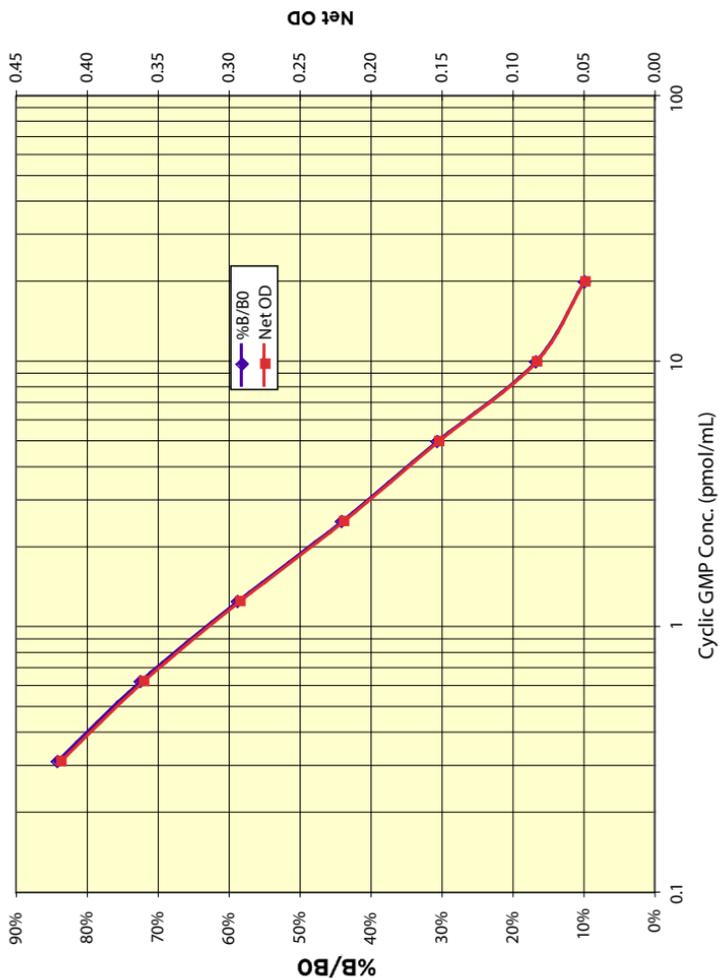
Typical Data - Acetylated Format

| Sample | Mean OD | Net OD | % B/B0 | Cyclic GMP Concentration (pmol/mL) |
|------------|---------|--------|--------|------------------------------------|
| NSB | 0.045 | 0 | - | - |
| Standard 1 | 0.107 | 0.062 | 12.5 | 20 |
| Standard 2 | 0.140 | 0.095 | 19.1 | 10 |
| Standard 3 | 0.192 | 0.147 | 29.6 | 5 |
| Standard 4 | 0.282 | 0.237 | 47.7 | 2.5 |
| Standard 5 | 0.367 | 0.322 | 64.8 | 1.25 |
| Standard 6 | 0.434 | 0.389 | 78.3 | 0.625 |
| Standard 7 | 0.474 | 0.429 | 86.3 | 0.3125 |
| B0 | 0.542 | 0.497 | 100.0 | 0 |
| Sample 1 | 0.231 | 0.186 | 37.4 | 1.820 |
| Sample 2 | 0.369 | 0.324 | 65.2 | 0.622 |

Always run your own standard curve for calculation of results.

Do not use this data.

Typical Standard Curve - Acetylated Format



Validation Data - Acetylated Format

Sensitivity and Limit of Detection - Acetylated

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

Sensitivity was determined as 0.188 pmol/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of acetylated zero standard and a low concentration acetylated human sample.

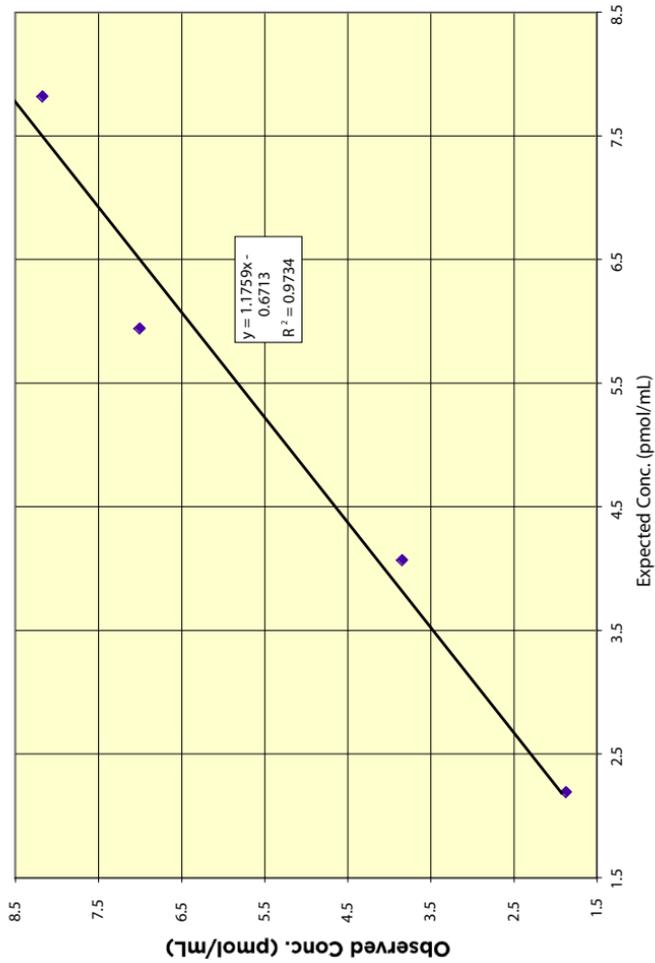
Limit of Detection was determined as 0.210 pmol/mL.

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% | 8.2 | 7.8 | 104.4 |
| 60% | 40% | 7.0 | 5.9 | 117.7 |
| 40% | 60% | 3.8 | 4.1 | 94.3 |
| 20% | 80% | 1.9 | 2.2 | 85.2 |
| | | | Mean Recovery | 100.4% |



Intra Assay Precision - Regular

Three human urine samples were diluted with Sample Diluent and run in replicates of 20 in an assay. The mean and precision of the calculated cGMP concentrations were:

| Sample | Cyclic GMP Concentration (pmol/mL) | %CV |
|--------|------------------------------------|------|
| 1 | 8.5 | 6.3 |
| 2 | 4.0 | 7.8 |
| 3 | 1.2 | 13.6 |

Inter Assay Precision - Regular

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

| Sample | Cyclic GMP Concentration (pmol/mL) | %CV |
|--------|------------------------------------|-----|
| 1 | 8.5 | 6.5 |
| 2 | 4.3 | 7.0 |
| 3 | 1.5 | 8.3 |

Intra Assay Precision - Acetylated

Three human urine samples were diluted with Sample Diluent, acetylated and run in replicates of 20 in an assay. The mean and precision of the calculated cGMP concentrations were:

| Sample | Cyclic GMP Concentration (pmol/mL) | %CV |
|--------|------------------------------------|-----|
| 1 | 8.1 | 3.8 |
| 2 | 3.8 | 5.2 |
| 3 | 1.2 | 8.2 |

Inter Assay Precision - Acetylated

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

| Sample | Cyclic GMP Concentration (pmol/mL) | %CV |
|--------|------------------------------------|------|
| 1 | 8.7 | 12.3 |
| 2 | 4.3 | 14.3 |
| 3 | 1.5 | 14.2 |

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 5.0 to 8.6 pmol/mL with an average for the samples of 7.16 pmol/mL. Seven normal human urine samples were diluted between 5 and 320 fold in Sample Diluent and values ranged in the neat samples from 19.9 to 3,305 pmol/mL with an average for the samples of 461.8 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% decreased measured cGMP by 9%. CTAC above 0.05% should not be used in the assay.

RESOURCES

References

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Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

Technical Service Contact Information

Phone: 250-294-9065

Fax: 250-294-9025

E-Mail: techsupport@stressmarq.com

Hours: M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

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NOTES

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