Product Manual

Guanosine Assay Kit

Catalog Number

MET-5149 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Guanosine is a purine nucleoside made up of a guanine moiety attached to a ribose ring through a β-N9-glycosidic bond. Guanosine can be converted to guanosine monophosphate (GMP), cyclic guanosine monophosphate (cGMP), guanosine diphosphate (GDP), and guanosine triphosphate (GTP) by the process of phosphoryation. These phosphorylated forms of guanosine play key roles in the processes of nucleic acid and protein synthesis, photosynthesis, muscle contraction, and intracellular signal transduction (specifically with cGMP).

Guanosine is required in mRNA for an RNA splicing reaction. When a "self-splicing" intron excises itself from the mRNA message (requiring a 5' guanosine) cutting at both ends, re-ligating, and leaving just the exons on either side to be translated into a functional protein. The antiviral drug acyclovir, which is used in the treatment of herpes, and the anti-HIV drug abacavir are both structurally similar to guanosine. Guanosine was also used to make regadenoson, an A2A adenosine receptor agonist that is a coronary vasodilator and is often used in pharmacologic stress testing. Guanosine prevents Quinolinic Acid (QA) induced seizures about as well as the NMDA receptor antagonist, MK-801. Besides acting as an NMDA receptor agonist, QA decreases glutamate transport/uptake. It has been shown that Guanosine counteracts the QA-induced decrease in glutamate uptake as well as increases in glutamate release from the synapse.

Cell Biolabs' Guanosine Assay Kit is a simple fluorometric assay that measures the amount of total Guanosine present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, Guanosine standards, and unknown samples. Sample Guanosine concentrations are determined by comparison with a known Guanosine standard. The kit has a detection sensitivity limit of 1.6 µM Guanosine.

Note: Each sample replicate requires 2 assays, one treated with purine nucleoside phosphorylase (+PNP) and one without (-PNP). Guanosine is calculated from the difference in RFU readings from the 2 wells.

Assay Principle

Cell Biolabs' Guanosine Assay Kit measures total Guanosine within biological samples. Guanosine is converted to Guanine by PNP. Guanine is converted into xanthine by guanine deaminase. Then xanthine is converted to uric acid and hydrogen peroxide by xanthine oxidase. The resulting hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of Guanosine standard within the 96-well microtiter plate format. Samples and standards are incubated for 15 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).

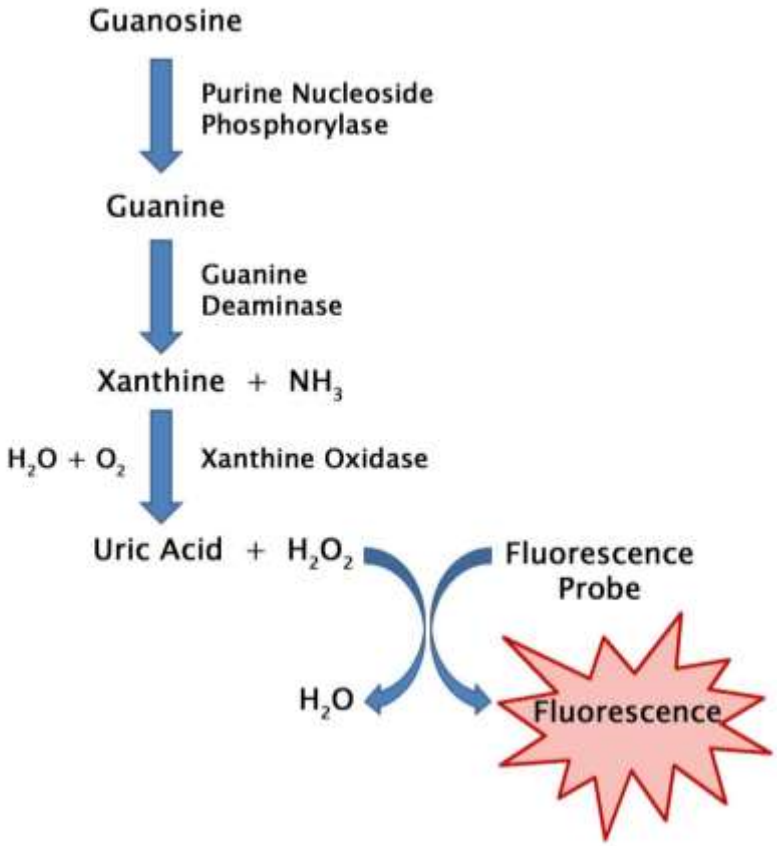

Figure 1. Guanosine Assay Principle.

Related Products

1. MET-5147: Guanine Assay Kit (Colorimetric)
2. MET-5148: Guanine Assay Kit (Fluorometric)
3. MET-5090: Adenosine Assay Kit
4. MET-5092: Inosine Assay Kit
5. STA-670: Homocysteine ELISA Kit

Kit Components

1. Guanosine Standard (Part No. 51491C): One 50 µL tube at 100 mM.
2. 10X Assay Buffer (Part No. 268002): One 25 mL bottle of 500 mM sodium phosphate pH 7.4.
3. Fluorometric Probe (Part No. 50231C): One 50 µL tube in DMSO.
4. HRP (Part No. 234402-T): One 10 µL tube of a 100 U/mL solution in glycerol.
5. 50X Guanine Deaminase (Part No. 51472D): One 100 µL tube.
6. Purine Nucleoside Phosphorylase (Part No. 50903D): One 500 µL tube at 18.9 U/mL

Note: One unit is defined as the amount of enzyme that will cause the phosphorolysis of 1.0 µmole of inosine to hypoxanthine and ribose 1-phosphate per minute at pH 7.4 at 25°C.

7. Xanthine Oxidase (Part No. 50904D): One 100 µL tube at 2.5 U/mL.

   Note: One unit is defined as the amount of enzyme that will convert 1.0 µmole of xanthine to uric acid per minute at pH 7.5 at 25°C. About 50% of the activity is obtained with hypoxanthine as substrate.

Materials Not Supplied

1. Phosphate Buffered Saline (PBS)
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate
5. Multichannel micropipette reservoir
6. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.

Storage

Upon receipt, store the 10X Assay Buffer at room temperature and store the rest of the kit at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Note: After thawing 50X Guanine Deaminase, Purine Nucleoside Phosphorylase, or Xanthine Oxidase for the first time, make smaller aliquots and store at -20°C.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, 50X Guanine Deaminase 1:50, Purine Nucleoside Phosphorylase 1:10, and Xanthine Oxidase 1:50 into 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, 20 µL of 50X Guanine Deaminase, 100 µL of Purine Nucleoside Phosphorylase, and 20 µL of Xanthine Oxidase to 848 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

- Control Mix: Prepare a Reaction Mix (without PNP) by diluting the Fluorometric Probe 1:100, HRP 1:500, 50X Guanine Deaminase 1:50, and Xanthine Oxidase 1:50 into 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, 20 µL of 50X Guanine Deaminase, and 20 µL of Xanthine Oxidase to 948 µL of 1X Assay Buffer for a total of 1 mL. This Control Mix volume is enough for 20 assays. The Control Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Cell culture supernatants: Cell culture media containing guanosine, guanine, xanthine, and hypoxanthine should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary in PBS.

  Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

- Tissue lysates: Sonicate or homogenize tissue sample in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant may be assayed directly or diluted as necessary in PBS.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in PBS.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in PBS.

  Notes:
  - *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
  - *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
  - *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Guanosine standards before use by diluting in PBS. First, dilute the stock Guanosine Standard 100 mM solution 1:10 in PBS for a 10 mM Guanosine Solution. (e.g. add 5 µL of the stock 100 mM Guanosine Standard to 45 µL of PBS; this 10 mM solution is stable at 4°C for one day). Use the 10 mM Guanosine Solution to prepare a series of the remaining Guanosine standards according to Table 1 below.

Standard Tubes	10 mM Guanosine Solution (µL)	PBS (µL)	Guanosine (µM)
1	5	495	100
2	250 of Tube #1	250	50
3	250 of Tube #2	250	25
4	250 of Tube #3	250	12.5
5	250 of Tube #4	250	6.25
6	250 of Tube #5	250	3.13
7	250 of Tube #6	250	1.57
8	0	250	0

Table 1. Preparation of Guanosine Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with Purine Nucleoside Phosphorylase (+PNP) and one without the enzyme (-PNP) to measure endogenous background.

2. Add 50 µL of each standard into wells of a black microtiter plate suitable for a fluorescence plate reader.
3. Add 50 µL of each unknown sample to each of two separate wells.
4. Add 50 µL of Reaction Mix to all standard wells and one half of the paired sample wells.
5. Add 50 µL of Control Mix to the remaining paired sample wells.
6. Mix the well contents thoroughly and incubate for 15 minutes at room temperature protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Purine Nucleoside Phosphorylase (-PNP) from the sample well values containing Purine Nucleoside Phosphorylase (+PNP) to obtain the difference. The fluorescence difference is due to the PNP activity.

$$\text{Net RFU} = (\text{RFU}_{+\text{PNP}}) - (\text{RFU}_{-\text{PNP}})$$

5. Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of Guanosine present in the sample. Only use values within the range of the standard curve.

Example of Results

The following figure demonstrates typical Guanosine Assay Kit (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

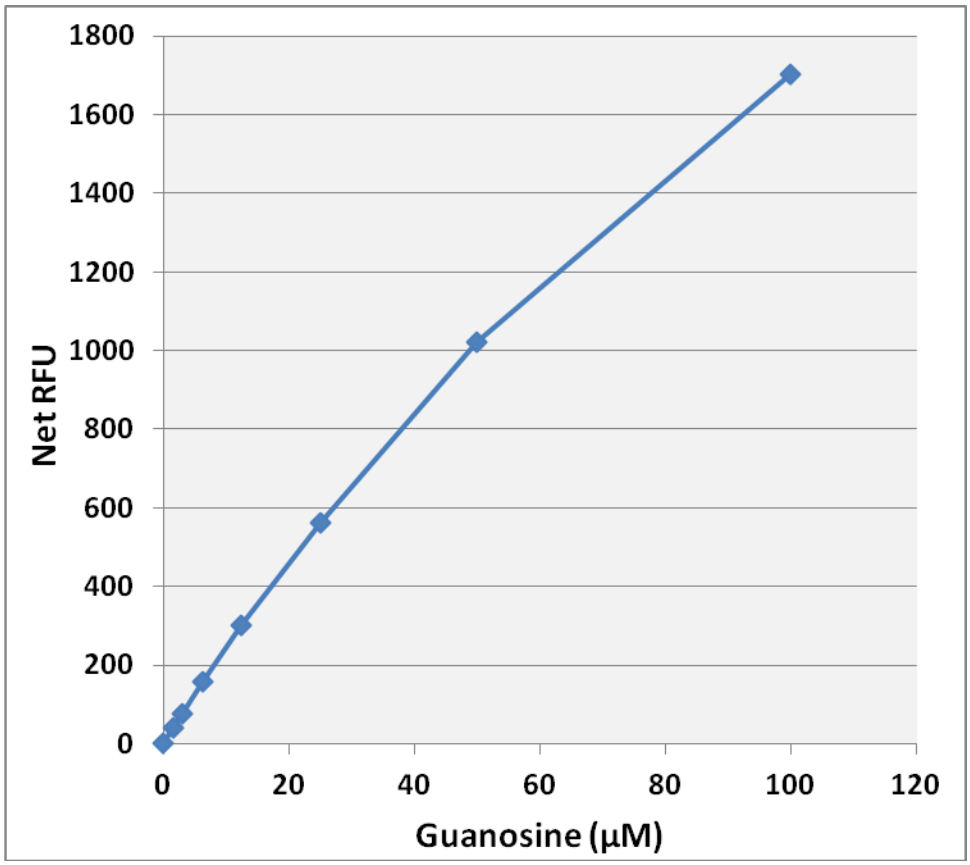

Figure 2: Guanosine Standard Curve.

References

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4. Tavares RG, Schmidt AP, Abud J, Tasca CI, Souza DO (2005). *Neurochem Res*. **30**: 439-444.
5. Tavares RG, Schmidt AP, Tasca CI, Souza DO (2008). *Neurochem Res*. **33**: 97-102.

Warranty

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