NOTE: Revision to Assay Protocol

Product Manual

Guanine Assay Kit (Colorimetric)

Catalog Number

MET-5147 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Guanine is one of the four common nucleobases of the nucleic acids deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In double stranded DNA, guanine forms base pairs with cytosine on the opposite strand, while RNA strands can also fold into secondary structure such as hairpin loops stabilized by the same guanine/cytosine base pairing. Binding occurs through three hydrogen bonds. Guanine is synthesized in cells by guanosine phosphorylase from guanosine and phosphate.

Guanine plays a number of biological roles including camouflage, display, and vision. In addition, some amphibians, spiders, and scorpions convert ammonia to guanine, the latter of which can be excreted with minimal loss of water. Interestingly, it has been reported based on NASA studies of meteorites containing guanine and other nucleobases that guanine can be formed extraterrestrial.

Cell Biolabs' Guanine Assay Kit is a simple colorimetric assay that measures the amount of total guanine present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, guanine standards, and unknown samples. Sample guanine concentrations are determined by comparison with a known guanine standard. The kit has a detection sensitivity limit of 3.1 µM guanine.

Note: Each sample replicate requires 2 assays, one treated with guanine deaminase (+GDA) and one without (-GDA). Guanine is calculated from the difference in OD readings from the 2 wells.

Assay Principle

This kit measures total guanine within biological samples. Guanine is converted into xanthine by guanine deaminase. Then xanthine is converted to uric acid and hydrogen peroxide by xanthine oxidase (XO). The resulting hydrogen peroxide is then detected with a highly specific colorimetric 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 guanine standard within the 96-well microtiter plate format. Samples and standards are incubated for 15 minutes and then read with a standard 96-well colorimetric plate reader (Figure 1).

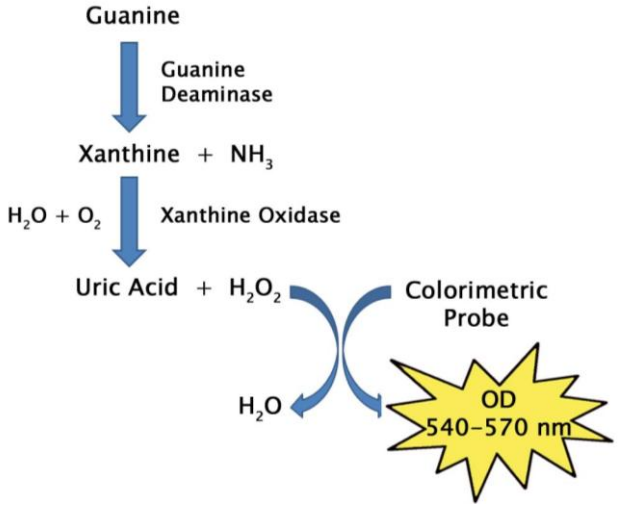

Figure 1. Guanine Assay Principle.

Related Products

1. MET-5014: NAD+/NADH Assay Kit (Colorimetric)
2. MET-5150: Xanthine/Hypoxanthine Assay Kit (Fluorometric)
3. STA-380: Global DNA Methylation Elisa Kit (Colorimetric)
4. MET-5070: Glycine Assay Kit (Fluorometric)
5. MET-5086: Ammonia Assay Kit (Colorimetric)

Kit Components (shipped on blue ice)

1. Guanine Standard (Part No. 51471C): One 50 µL tube at 20 mM.
2. 10X Assay Buffer (Part No. 268002): One 25 mL bottle of 500 mM sodium phosphate pH 7.4.
3. Colorimetric Probe (Part No. 50222C): 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. 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 min 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. Standard 96-well clear microtiter plate and/or clear cell culture microplate

Storage

Upon receipt, store the 10X Assay Buffer at room temperature and store the rest of the kit at -20ºC. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.
Note: After thawing 100X Guanine Deaminase 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 Colorimetric Probe 1:100, HRP 1:500, Guanine Deaminase 1:50, and Xanthine Oxidase 1:50 into 1X Assay Buffer. For example, add 10 µL Colorimetric Probe stock solution, 2 µL HRP stock solution, 20 µL of Guanine Deaminase, and 20 µL of Xanthine Oxidase to 948 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 guanine deaminase) by diluting the Colorimetric Probe 1:100, HRP 1:500, and Xanthine Oxidase 1:50 in 1X Assay Buffer. For example, add 10 µL Colorimetric Probe stock solution, 2 µL HRP stock solution, and 20 µL of Xanthine Oxidase to 968

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 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 Colorimetric 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 Colorimetric 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 Colorimetric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Guanine standards according to Table 1.

Standard Tubes	20 mM Guanine Solution (µL)	PBS (µL)	Guanine (µM)
1	5	495	200
2	250 of Tube #1	250	100
3	250 of Tube #2	250	50
4	250 of Tube #3	250	25
5	250 of Tube #4	250	12.5
6	250 of Tube #5	250	6.25
7	250 of Tube #6	250	3.13
8	0	250	0

Table 1. Preparation of Guanine 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 Guanine Deaminase (+GDA) and one without the enzyme (-GDA) to measure endogenous background.

2. Add 50 µL of each standard into wells of a microtiter plate suitable for a colorimetric 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 60 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 spectrophotometric microplate reader in the 540-570 nm range.

Example of Results

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

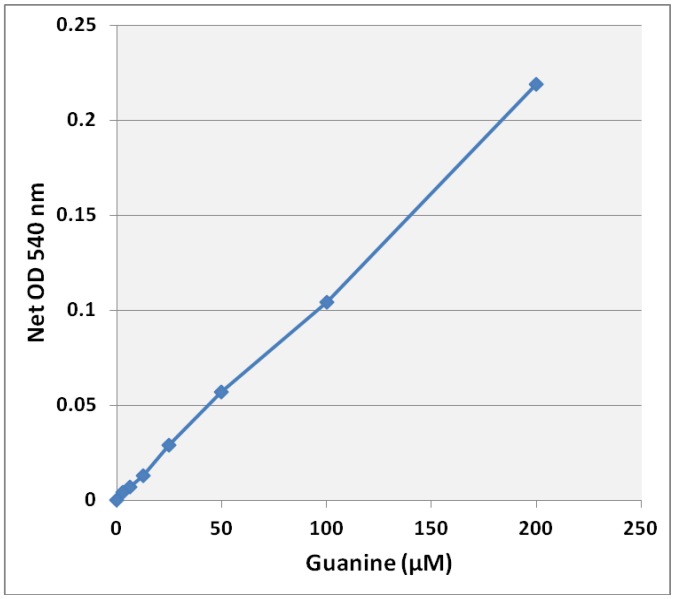

Figure 2: Guanine Standard Curve.

Calculation of Results

1. Determine the average absorbance 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 Guanine Deaminase (-GDA) from the sample well values containing Guanine Deaminase (+GDA) to obtain the difference. The absorbance difference is due to the Guanine Deaminase activity.

$$\Delta OD = (OD_{+GDA}) - (OD_{-GDA})$$

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

References

1. Shafer RH (1997). *Prog. Nuc. Acid. Res. Mol. Biol.* **59**: 55-94
2. Votyakova TV, and Reynolds IJ (2001) *Neurochem*. **79**:266.
3. Gur D, Palmer BA, Weiner, S, and Addadi L (2017) *Adv. Func. Mat.* **27**:1-13
4. Callahan, Smith KE, Cleaves HJ, Ruzica J, Stern JC, Glavin DP, House CH, and Dworkin JP (2011) *Proc Natl Acad Sci*. **108**: 13995-13998.

Recent Product Citation

De Boni, L. et al. (2026). Placing Purines in Precision Medicine: Targeting a Metabolic Reliance in KRAS-Mutant Tumors. *iScience*. doi: 10.1016/j.isci.2026.114954.

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