Guanosine Assay Kit

ARG83593 Guanosine Assay Kit is a detection kit for the quantification of Guanosine.

Catalog number: ARG83593

Package: 96 wells

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PRINCIPLE OF THE ASSAY

ARG83593 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store **10x Assay Buffer** at **RT**, **other component** store at **-20°C**. Use the kit before expiration date.

Component	**Quantity**	**Storage information**
Guanosine Standard (100 mM Guanosine)	50 µl	-20°C
10x Assay Buffer	25 ml	4°C
50x Guanosine Deaminase	100 µl	-80°C
50x Xanthine Oxidase	100 µl	-20°C
100x Probe	50 µl	-20°C
500x HRP	10 µl	-20°C
Xanthine Oxidase	100 µl	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader
- Pipettes and pipette tips
- Deionized or distilled water
- Microscope

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store **10x Assay Buffer** at **RT**, **other component** store at **-20°C**. Use the kit before expiration date.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine- Collect the first urine of the day, micturate directly into a sterile container. Remove impurities by centrifugation. Collect the supernatants and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Cell or Tissue Lysate- Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect samples and assay immediately or aliquot and store samples at-80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1x Assay Buffer** - Dilute the **10x Assay Buffer** into Deionized Water to yield **1X Assay Buffer**. The **1x Assay Buffer** is stable for up to 6 months at 2-8°C.
- **Working Detection Reagent**- Prepare this reagent immediately prior to use and use it within 20 min after preparation. Probe 1:100, HRP 1:500, 50X Guanine Deaminase 1:50, Purine Nucleoside Phosphorylase 1:10, and Xanthine Oxidase 1:50 in 1X Assay Buffer.
- **Working Control Reagent**- Prepare this reagent immediately prior to use and use it within 20 min after preparation. Probe 1:100, HRP 1:500, 50X Guanine Deaminase 1:50, and Xanthine Oxidase 1:50 in 1X Assay Buffer.
- **Standards:** Prepare fresh Lysine Standards before use by diluting in 1X Assay Buffer according to the Table below.

Standard tube	Guanosine (µM)	1X Assay Buffer (µL)	Standard (µL)
S1	100	495	5 (20 mM Guanosine Standard Stock)
S2	50	250	250 of S1
S3	25	250	250 of S2
S4	12.5	250	250 of S3
S5	6.25	250	250 of S4
S6	3.13	250	250 of S5
S7	1.57	250	250 of S6
S0	0	200	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add 50 µl of diluted samples or each diluted Standard into respective wells of the 96-well plate.
2. Add 50 µl of Working Detection/Control Reagent to each well.
3. Cover the plate and incubate for 15 minutes at **RT**.
4. Read the absorbance with a plate reader at O.D. 530-570 nm.

CALCULATION OF RESULTS

- Plot the RFU measured at 15 minutes for each standard against the standard concentrations. Determine the slope using linear regression fitting. The Guanosine concentration of a Sample is calculated as follow:

  Net RFU = (RFU_{+PNP})- (RFU_{-PNP})

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Guanosine. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.