Inosine Assay kit

ARG83373 Inosine Assay kit is an assay kit for Inosine in Serum, plasma, urine, Cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83373

Package: 100 assay

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INTRODUCTION

Inosine is a nucleoside that is formed when hypoxanthine is attached to a ribose ring (also known as a ribofuranose) via a β-N9-glycosidic bond. It was discovered in 1965 in analysis of RNA transferase.[1] Inosine is commonly found in tRNAs and is essential for proper translation of the genetic code in wobble base pairs.

Knowledge of inosine metabolism has led to advances in immunotherapy in recent decades. Inosine monophosphate is oxidised by the enzyme inosine monophosphate dehydrogenase, yielding xanthosine monophosphate, a key precursor in purine metabolism. Mycophenolate mofetil is an anti-metabolite, anti-proliferative drug that acts as an inhibitor of inosine monophosphate dehydrogenase. It is used in the treatment of a variety of autoimmune diseases including granulomatosis with polyangiitis because the uptake of purine by actively dividing B cells can exceed 8 times that of normal body cells, and, therefore, this set of white cells (which cannot operate purine salvage pathways) is selectively targeted by the purine deficiency resulting from inosine monophosphate dehydrogenase (IMD) inhibition.

PRINCIPLE OF THE ASSAY

ARG83373 Inosine Assay Kit measures Inosine content in biological samples. Samples are compared to a known concentration of Inosine standard within the 96-well microtiter plate format. The intensity of the color is measured at a wavelength of 590 nm. The concentration of Inosine in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 20X Assay Buffer at RT.

Store other component at ≤ -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (2.0 mM)	50 µL	-20°C
10X Assay Buffer	25 ml	RT
Purine Nucleoside Phosphorylase	500 µL	-20°C
Xanthine Oxidase	100 µL	-20°C
Probe	50 µL	-20°C
HRP-Streptavidin Solution	10 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 590 nm
- Flat bottomed 96-well black microplate and tube.
- 1X PBS and deionized water
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Store 10X Assay Buffer at RT. Store other component at ≤ -20°C. Use the kit before expiration date. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

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

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 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 urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. 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.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants 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.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at RT.

- **Reaction Mix:** Dilute the Probe at 1:100, HRP-Streptavidin Solution at 1:500, Purine Nucleoside Phosphorylase at 1:10 and Xanthine Oxidase at 1:50 in 1X Assay Buffer. For 20 assays, add 10 µL Probe stock solution, 2 µL HRP-Streptavidin Solution, 100 µL of Purine Nucleoside Phosphorylase and 20 µL of Xanthine Oxidase to 868 µL of 1X Assay Buffer for a total of 1 mL. Store the Reaction Mix at 4°C for 1 day.
- **Control Mix:** Dilute the Probe at 1:100, HRP-Streptavidin Solution at 1:500 and Xanthine Oxidase at 1:50 in 1X Assay Buffer. For 20 assays, add 10 µL Probe stock solution, 2 µL HRP-Streptavidin Solution and 20 µL of Xanthine Oxidase to 968 µL of 1X Assay Buffer for a total of 1 mL. Store the Reaction Mix at 4°C for 1 day.

- **Standards**: Add 5 µl of 2.0 mM stock standard into 495 µl PBS to generate a standard with 20 µM of Tyrosine. Dilute the standards with PBS serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	Inosine(µM)	Volume of PBS (µL)	Volume of Inosine(µL)
S1	20	495	5 (2.0 mM stock)
S2	10	250	250(S1)
S3	5	250	250(S2)
S4	2.5	250	250(S3)
S5	1.25	250	250(S4)
S6	0.625	250	250(S5)
S7	0.313	250	250(S6)
S8	0	250	0

ASSAY PROCEDURE

Each samples should be assayed in at least duplicates, one to be treated with Purine Nucleoside Phosphorylase and one without, to measure endogenous background.

1. Add 50 µl of standard and sample to each wells.
2. Add 50 µL of Reaction Mix to standard and half of sample wells.
3. Add 50 µL of Control Mix to other half of sample wells.
4. Mix well and Incubate for 15 min at RT.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.
5. Read O.D. with a microplate reader at 590 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average value of Standard 0 from all standard value.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

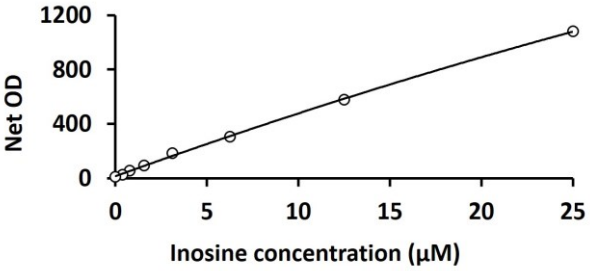
4. Subtract the sample well values without Purosine Nucleoside Phosphorylase from the sample well values containing enzyme (Control Mix) to obtain the difference. The absorbance difference is due to the Purosine Nucleoside Phosphorylase activity:

   $\Delta A = A_{\text{Reaction Mix}} - A_{\text{Control Mix}}$

5. Compare the change in absorbance Δ A of each sample to the standard curve to determine and extrapolate the quantity of bile acid present in the sample. Only use values within the range of the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Inosine ranged from 0.313-20 µM. The mean MDD was 0.1 µM