Product Manual

Inosine Assay Kit

Catalog Number

MET-5092 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Inosine is a nucleoside that is created when a ribose ring attaches to hypoxanthine through a β-N9-glycosidic bond. In the DNA synthesis pathway, adenine is first modified to form adenosine or inosine monophosphate (IMP) Next, either form is converted into inosine which can form base pairs with adenine (A), cytosine (C), and uracil (U). Inosine is most often found in tRNAs and is important for faithful translation of the genetic code in wobble base pairs.

A better understanding of inosine metabolism has led to immunotherapy advances in recent years. Inosine monophosphate is oxidized by inosine monophosphate dehydrogenase to create xanthosine monophosphate, an important precursor in purine metabolism. Mycophenolate mofetil is a drug that acts as an inhibitor of inosine monophosphate dehydrogenase and is used in the treatment of a number of autoimmune diseases including granulomatosis with polyangiitis. In addition inosine has been demonstrated to have neuroprotective properties. It has been suggested for administration in both spinal cord injury and after stroke since it enhances the rewiring of axonal connections. Inosine may also benefit multiple sclerosis (MS) patients since ingestion leads to conversion to uric acid that is thought to be a natural antioxidant and a peroxynitrite scavenger. Inosine treatment of Parkinson's disease patients has been shown to slow progression of the disease in clinical trials.

Cell Biolabs' Inosine Assay Kit is a simple fluorometric assay that measures the amount of total inosine present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, inosine standards, and unknown samples. Sample inosine concentrations are determined by comparison with a known inosine standard. The kit has a detection sensitivity limit of 300 nM inosine.

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

Assay Principle

Cell Biolabs' Inosine Assay Kit measures total inosine within biological samples. Inosine is converted into hypoxanthine by purine nucleoside phosphorylase (PNP). Then hypoxanthine is converted to xanthine and hydrogen peroxide by xanthine oxidase (XO). 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 inosine 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).

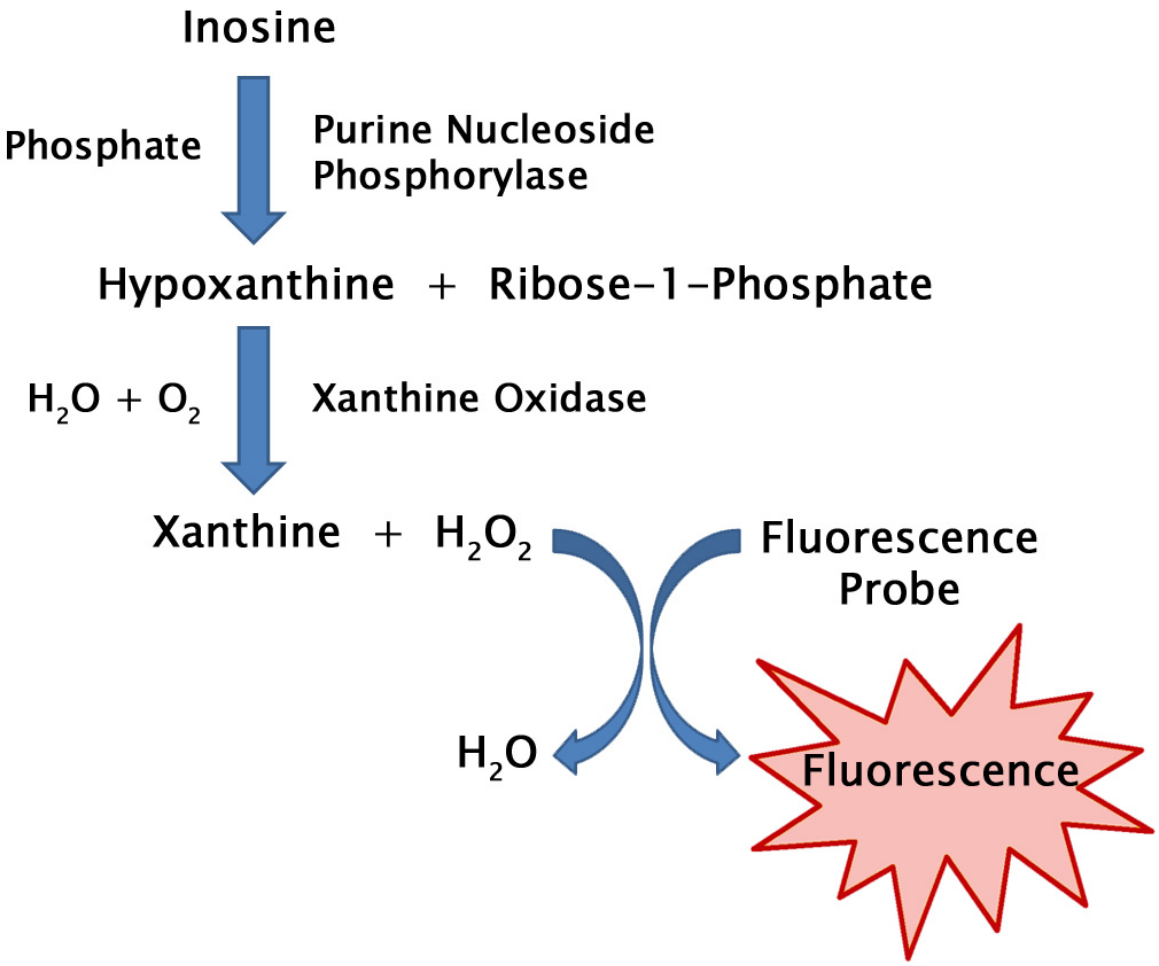

Figure 1. Inosine Assay Principle.

Related Products

1. MET-5090: Adenosine Assay Kit
2. PRB-5047: Human CK-MB ELISA Kit (Creatine Kinase-MB)
3. PRB-5050: Human Cardiac Troponin I (cTnI) ELISA Kit
4. STA-392: Human C-Reactive Protein (CRP) ELISA Kit
5. STA-670: Homocysteine ELISA Kit
6. STA-671: S-Adenosylhomocysteine (SAH) ELISA Kit
7. STA-672: S-Adenosylmethionine (SAM) ELISA Kit

Kit Components

1. Inosine Standard (Part No. 50921C): One 50 µL tube at 2 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. 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 min at pH 7.4 at 25 °C.
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. 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 any of the three enzymes 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, Purine Nucleoside Phosphorylase 1:10, and Xanthine Oxidase 1:1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock 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. 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 purine nucleoside phosphorylase) by diluting the Fluorometric Probe 1:100, HRP 1:500, and Xanthine Oxidase 1:1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric 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 inosine, 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 Inosine standards according to Table 1 below.

Standard Tubes	2 mM Inosine Solution (µL)	PBS (µL)	Inosine (µM)
1	5	495	20
2	250 of Tube #1	250	10
3	250 of Tube #2	250	5
4	250 of Tube #3	250	2.5
5	250 of Tube #4	250	1.25
6	250 of Tube #5	250	0.625
7	250 of Tube #6	250	0.313
8	0	250	0

Table 1. Preparation of Inosine 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 Purosine Nucleoside Phosphorylase (-PNP) from the sample well values containing Purosine 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 Inosine present in the sample. Only use values within the range of the standard curve.

Example of Results

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

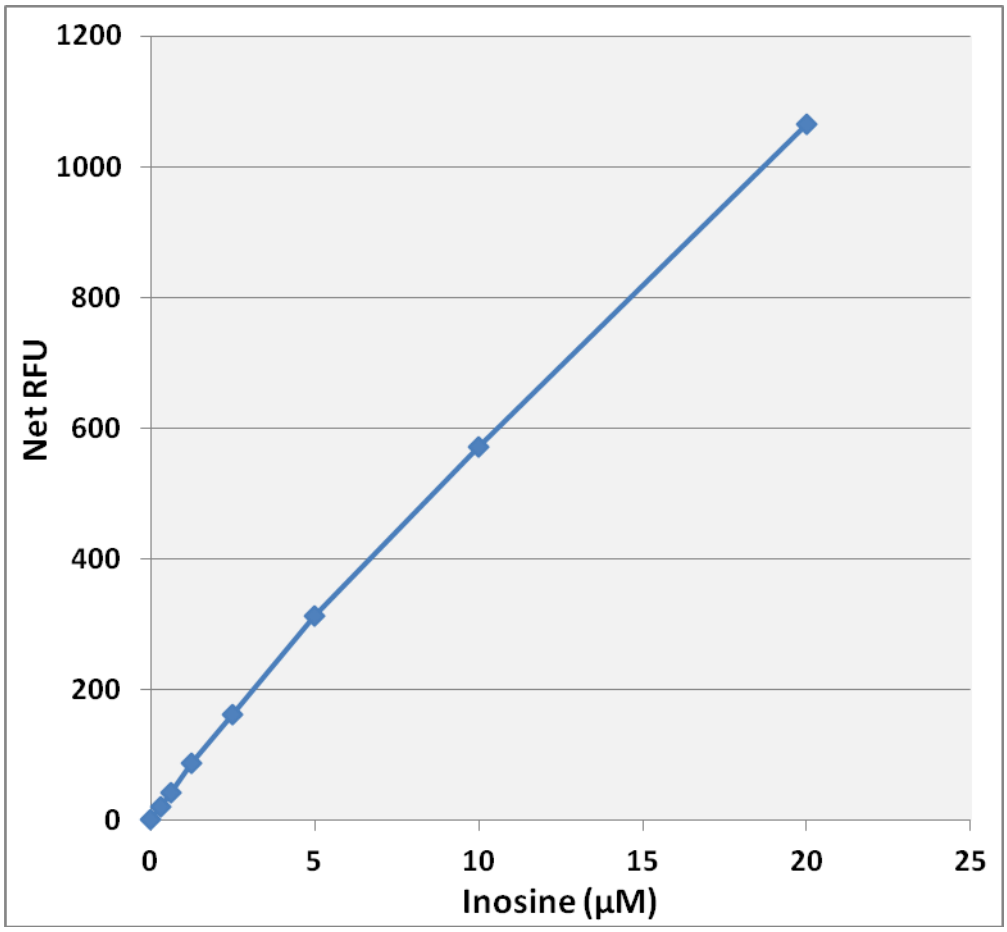

Figure 2: Inosine Standard Curve.

References

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Warranty

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com