EnzyFluo™ Inosine Assay Kit (EINS-100)

Quantitative Fluorimetric Inosine Concentration Determination

DESCRIPTION

INOSINE is a purine nucleoside formed from Ribose and Hypoxanthine that can be found in most animal tissues and fluids. Inosine is most often found in tRNAs and is important for proper translation of the genetic code. It may play a role in cancer immunometabolism. It is degraded via the purine degradation pathway, first by purine nucleoside phosphorylase to hypoxanthine, then to xanthine by xanthine oxidase and then finally to uric acid by further action of xanthine oxidase.

Simple, direct and high-throughput assays for measuring inosine find wide applications in research and drug discovery. BioAssay Systems' inosine assay kit uses a single Working Reagent that combines the enzyme reactions and color reaction in one step. The change in fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to inosine concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 20 µL of sample. Linear detection range in 96-well plate for 30-minute incubation with 1 to 25 µM.

Simple and convenient. The procedure involves addition of a single working reagent and incubation for 30 min at room temperature.

Fast and high-throughput. Assays using 96-well plates and liquid handling system could allow simultaneous processing tens of thousands of samples per day.

APPLICATIONS

Direct Assays: inosine concentration in cell lysate, serum, and other biological samples.

Drug Discovery/Pharmacology: effects of drugs on inosine (purine) metabolism.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer:	10 mL	**HRP Enzyme:**	120 µL
Standard:	100 µL 1 mM Inosine	**Dye Reagent:**	120 µL
XO Enzyme:	100 µL	**PNP Enzyme:**	100 µL

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURE

Samples can be analyzed immediately after collection or stored in aliquots at –20°C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

1. Equilibrate all components to room temperature. During the experiment, keep all the enzymes in a refrigerator or on ice.

2. *Standard Curve*. Prepare a 25 µM stock of Inosine by mixing 10 µL of 1 mM Inosine standard with 390 µL of dH_2O. Create a standard curve as shown in the Table below.

No	Standard + H_2O	Vol (µL)	Inosine (µM)
1	100 µL + 0 µL	100	25
2	60 µL + 40 µL	100	15
3	30 µL + 70 µL	100	7.5
4	0 µL + 100 µL	100	0

Transfer 20 µL standards into separate wells of a black, flat bottom 96-well plate

3. *Sample Preparation*. Hypoxanthine and Xanthine interfere with this reaction. If a sample is known to contain either or both Hypoxanthine and Xanthine, prepare two separate 20 µL aliquots of each sample and transfer into separate wells. One will serve as the Sample (+ PNP enzyme), and one will serve as the Sample Blank (- PNP enzyme).

4. *Working Reagent*. For each sample and standard well, prepare Working Reagent by mixing 80 µL Assay Buffer, 1 µL XO Enzyme, 1 µL PNP Enzyme, 1 µL HRP Enzyme, and 1 µL Dye Reagent in a clean tube.

   Where a sample blank is required, prepare Sample Blank Working Reagent (- PNP enzyme) by mixing 80 µL Assay Buffer, 1 µL XO Enzyme, 1 µL HRP Enzyme, and 1 µL Dye Reagent per reaction well in a clean tube.

   Transfer 80 µL Working Reagent into sample and standard wells, and 80 µL of Blank Working Reagent into the sample blank wells. Tap plate to mix.

5. Incubate 30 min at room temperature, and then read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

Subtract water (#4) fluorescence from fluorescence values for the standards. Plot ΔF against standard concentrations. Determine the slope and calculate sample inosine concentration,

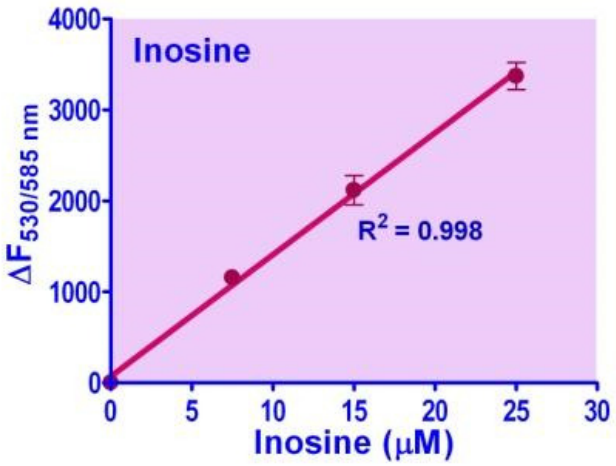
$$[\text{Inosine}] = \frac{F_{SAMPLE} - F_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

Where F_{Sample} and F_{Blank} are the fluorescence values of the sample and water (if sample does not contain Hypoxanthine and Xanthine) or sample blank (if sample contains Hypoxanthine and Xanthine). *Slope* is the slope of the standard curve and *n* is the dilution factor.

Notes: If the calculated sample concentration is higher than 25 µM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor (*n*).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, black 96-well plates (e.g. Greiner Bio-One, cat# 655900) and plate reader capable of measuring fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm.



LITERATURE

1. Dominissini D et al (2011) Adenosine-to-Inosine RNA editing meets cancer. Carcinogenesis. 32(11):1569-77.
2. Samami E et al (2023) Inosine, gut microbiota, and cancer immunometabolism. Am J Physiol Endocrinol Metab. 324 (1): E1-E8.
3. Srinivasan S, Torres AG, Ribas de Pouplana L (2021) Inosine in Biology and Disease. Genes (Basel). 12(4):600