Technical Bulletin

Inosine Assay Kit

Catalogue Number MAK627

Product 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 metabolism. 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.

The 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 λex/em = 530/585 nm is directly proportional to inosine concentration in the sample. The linear detection range for a 30-minute incubation is 1 to 25 µM.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK627A
- HRP Enzyme 120 µL
  Catalogue Number MAK627B
- XO Enzyme 100 µL
  Catalogue Number MAK627C
- PNP Enzyme 100 µL
  Catalogue Number MAK627D
- Dye Reagent 120 µL
  Catalogue Number MAK627E
- Standard (1 mM) 100 µL
  Catalogue Number MAK627F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescent multiwell plate reader capable of λex/em = 530/585 nm.
- Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent). Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store at -20°C upon receipt.

Preparation Instructions

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.

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).

Standard Preparation

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.

Table 1.
Standard Preparation

Std #	Standard Premix (µL)	Water (µL)	Conc. (µM)
1	100	0	25
2	60	40	15
3	30	70	7.5
4	0	100	0

Procedure

Assay Reaction

1. Transfer 20 µL of sample and standards into separate wells of a black, flat bottom 96-well plate.
2. 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.
3. 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.
4. 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.
6. Read fluorescence at λex/em = 530/585 nm.

Results

Calculations

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
n is the dilution factor

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

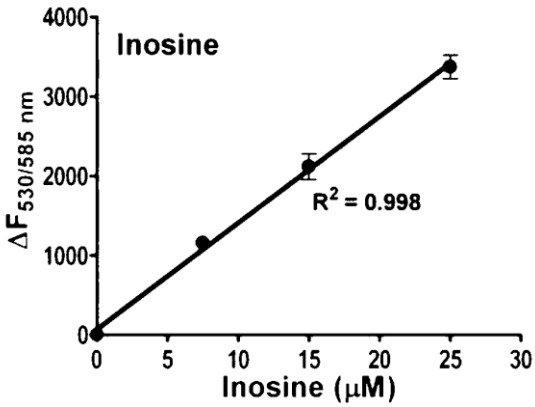

Figure 1.
Exemplary standard curve

References

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

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