3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Inosine Assay Kit

Catalog Number **MAK100**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Inosine is a purine nucleotide found at the wobble position of tRNA where it plays a role in the proper translation of mRNA at the ribosome. In addition to its role in translation, inosine also plays important roles in the immune system where is exhibits both inflammatory and anti-inflammatory effects. Inosine may be tissue protective during ischemic injury and recent reports suggest inosine may preserve cell viability during hypoxia.

The Inosine Assay Kit provides a simple and direct procedure for measuring inosine in a variety of samples. Inosine concentration is determined by a coupled enzyme reaction in which inosine is converted to hypoxanthine which reacts with the substrate mix and probe, resulting in a fluorometric product (λ_{ex} = 535 nm/λ_{em} = 587 nm), proportional to the inosine present.

Components

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

Inosine Assay Buffer Catalog Number MAK100A	25 mL
Inosine Probe Catalog Number MAK100B	0.4 mL
Converter Enzyme Catalog Number MAK100C	1 vl
Developer Enzyme Catalog Number MAK100D	1 vl
Inosine Substrate Mix Catalog Number MAK100E	1 vl
Inosine Standard, 10 mM Catalog Number MAK100F	50 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader
- 60% Perchloric acid (Catalog Number 311413 or equivalent)
- Potassium carbonate (Catalog Number P5833 or equivalent)
- Sodium Hydroxide (Catalog Number S8045 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Inosine Assay Buffer – Allow buffer to come to room temperature before use.

Converter Enzyme, Developer Enzyme, Inosine Substrate Mix – Reconstitute each in 220 µL of Inosine Assay Buffer. Mix well by pipetting, then aliquot each and store, protected from light at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Inosine Standards for Fluorometric Detection
Dilute 5 µL of the 10 mM (10 nmole/µL) Inosine Standard Solution with 995 µL of Inosine Assay Buffer to prepare a 50 µM (0.05 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 50 µM Inosine standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add Inosine Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid samples like serum and plasma can be measured directly.

Tissue (10–100 mg) or cells (5×10^6) can be homogenized in 0.7 mL of ice-cold 0.4 M perchloric acid. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Transfer the supernatant to a new tube and then neutralize by adding 10 µL of 4 M potassium carbonate solution per 100 µL volume. Incubate on ice for 7–10 minutes. Centrifuge the neutralized supernatant at 13,000 × *g* for 10 minutes. Add samples to wells or freeze at –80 °C until ready to analyze.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

If desired, the pellet from the perchloric acid homogenization step can be dissolved in 300 µL of 0.5 M sodium hydroxide solution and used for total protein analysis.

Bring samples to a final volume of 50 µL with Inosine Assay Buffer.

Notes: Xanthine, hypoxanthine, and NADH in the samples can generate a background signal. To remove the effect of background, a sample blank may be set up for each sample by omitting the Converter Enzyme from the reaction mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Inosine Assay Buffer	40 µL	42 µL
Inosine Probe	4 µL	4 µL
Inosine Substrate Mix	2 µL	2 µL
Converter Mix	2 µL	–
Developer Mix	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Cover the plate and protect from light during the incubation.

3. Measure fluorescence intensity (λ_{ex} = 535 nm/ λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Inosine Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Inosine standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the sample blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Inosine present in the samples may be determined from the standard curve.

Concentration of Inosine

$$S_a/S_v = C$$

S_a = Amount of Inosine in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of Inosine in sample

Inosine molecular weight: 268.23 g/mole

Sample Calculation

Amount of Inosine (S_a) = 0.58 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Inosine in sample

$$0.58 \text{ nmole}/50 \text{ μL} = 0.0116 \text{ nmole/μL}$$

$$0.1168 \text{ nmole/μL} \times 268.23 \text{ ng/nmole} = 3.11 \text{ ng/μL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

LS,MAM 02/14-1