Inosine Fluorometric Assay Kit

(Catalog #K712-100; 100 Assays; Store kit at -20°C)

I. Introduction:

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 and hypoxanthine are also potential markers of ATP catabolic-by-products from oxidative stress which follows acute cardiac ischemia. In BioVision's Inosine Assay Kit, inosine is converted to hypoxanthine, which will react with the substrate mix and PicoProbe™ to generate fluorescence (Ex/Em = 535/587 nm) in presence of converter and developer enzymes. This assay has a detection limit of of approximately 100 pmol inosine/well.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Inosine Assay Buffer	25 ml	WM	K712-100-1
PicoProbe™	0.4 ml	Blue	K712-100-2
Converter Enzyme Mix (lyophilized)	1 vial	Green	K712-100-3
Developer Enzyme Mix (lyophilized)	1 vial	Brown	K712-100-4
Inosine Substrate Mix (lyophilized)	1 vial	Red	K712-100-5
Inosine Standard (10 mM)	50 µl	Yellow	K712-100-6

III. Storage and Handling:

Store kit at –20°C, protect from light. Allow reagents to warm to room temperature and briefly centrifuge vials prior to opening. Read the entire protocol before the assay.

IV. Reagent Preparation:

Converter Enzyme Mix, Developer Enzyme Mix, Inosine Substrate Mix: Dissolve in 220 µl Assay Buffer separately. Aliquot and store at -20°C. Avoid multiple freeze/thaw cycles. Use within two months.

V. Inosine Assay Protocol:

1. Standard Curve Preparations:

Dilute the Inosine Standard to 50 µM by adding 5 µl of the Inosine Standard to 995 µl of Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of the Inosine Standard.

2. Sample Preparations:

Liquid samples can be assayed directly. For tissue or cell samples use 10 - 100 mg tissue or 5 million cells and homogenize in 0.7 ml of ice-cold 0.4 M perchloric acid then pellet for 5-10 min at 10 K x g. Collect the supernatant and neutralize with 10 µl of ice-cold 4 M K_2CO_3 per 100 µl volume; keep on ice for 7-10 min. The pellets can be dissolved in 300 µl of 0.5 N NaOH and used for total protein analysis. Centrifuge the neutralized supernatant again as above and immediately freeze at -80°C until ready to analyze. Add 1 - 50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer. For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

***Note:** Xanthine, Hypoxanthine and NADH in the sample will interfere with Inosine Assay. If significant amount of them are in your sample, include a sample background control by omitting the Converter Enzyme in the reaction mix.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

Reaction Mix		Sample Background Control	
40 µl	Assay Buffer	42 µl	Assay Buffer
4 µl	PicoProbe™	4 µl	PicoProbe™
2 µl	Substrate Mix	2 µl	Substrate Mix
2 µl	Converter Enzyme	-----	
2 µl	Developer Enzyme	2 µl	Developer Enzyme

Add 50 µl of the Reaction Mix to each well containing the Inosine Standard and test samples, and add 50 µl Sample Background Control to each background control well; mix well.

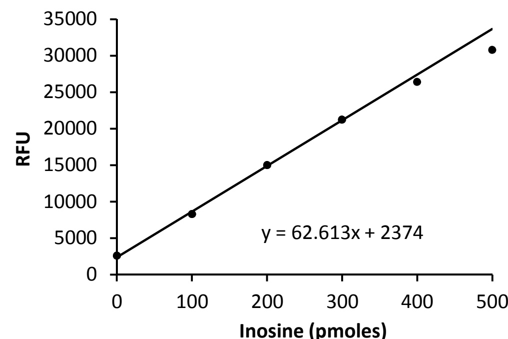
4. Incubate: The reaction for 30 minutes at 37°C, protected from light.

5. Measurement: Read the fluorescence at Ex/Em = 535/587 nm in a micro plate reader.

6. Calculation: Correct background by subtracting the value derived from the Sample Background Control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Subtract the 0 Inosine Standard from all Standards. Plot the Inosine Standard Curve. Apply the sample readings to the standard curve to calculate Inosine Concentration (C):

$$C = (S_a/S_v) \text{ nmol/µl or mM}$$

Where: S_a is the sample amount of unknown (in nmol) from standard curve,
S_v is sample volume (µl) added to the wells.
Inosine MW is 268.23 g/mol



RELATED PRODUCTS:

Glutathione Assay Kit	Myeloperoxidase (MPO) Colorimetric Assay Kit
Lipid Peroxidation (MDA) Kit	Myeloperoxidase (MPO) Fluorometric Assay Kit
Hydrogen Peroxide Assay Kit	Uric Acid Assay Kit
GST Colorimetric Assay Kit	Xanthine Oxidase Activity Assay Kit
GST Fluorometric Assay Kit	Nitric Oxide Colorimetric Assay Kit
Thioredoxin Reductase Activity Kit	Nitric Oxide Fluorometric Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		