ab126286

Inosine Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Inosine levels in various samples

This product is for research use only and is not intended for diagnostic use.

Version: 5b Last updated: 4 July 2025

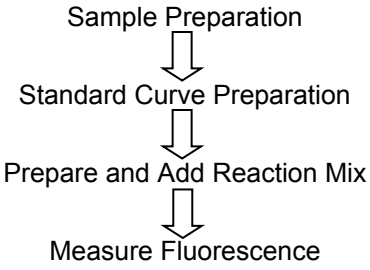
Table of Contents

1. Overview

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 ab126286, inosine is converted to hypoxanthine, which will react with the Substrate Mix I and PicoProbe I to generate fluorescence (Ex/Em = 535/587 nm) in presence of converter and Developer Mix J. This assay has a detection limit of of approximately 100 pmol inosine/well.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 50	25 mL
PicoProbe I	0.4 mL
Converter Mix E	1 vial
Developer Mix J	1 vial
Substrate Mix I	1 vial
Inosine Standard	50 µL

PLEASE NOTE: Developer Mix J was previously labelled as Developer Mix IV and Developer Enzyme Mix (Lyophilized), and Assay Buffer 50 as Assay Buffer L and Inosine Assay Buffer, and Substrate Mix I as Inosine Substrate Mix (Lyophilized), and Converter Mix E as Converter Enzyme VI and Converter Enzyme Mix (Lyophilized) and PicoProbe I as PicoProbe. The composition has not changed.

* 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.

Converter Mix E, Developer Mix J, and Substrate Mix I: Dissolve in 220 µl Assay Buffer 50 separately. Aliquot and store at -20°C. Avoid multiple freeze/thaw cycles. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- 0.4M Perchloric acid
- 4M Potassium carbonate
- 0.5M Sodium hydroxide
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

For tissue or cell samples:

a) 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 10,000 x *g*.

b) Collect the supernatant and neutralize with 10 µl of ice cold 4 M potassium carbonate per 100 µl volume; keep on ice for 7-10 min. The pellets can be dissolved in 300 µl of 0.5 N sodium hydroxide and used for total protein analysis.

c) Centrifuge the neutralized supernatant again as above and immediately freeze at -80°C until ready to analyze.

d) Add 1-50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer 50.

Liquid samples can be assayed directly. 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 Mix E in the reaction mix.

2. Standard Curve Preparation:

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

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	**Bkgd Control Mix**
Assay Buffer 50	40 µl	42 µl
PicoProbe I	4 µl	4 µl
Substrate Mix I	2 µl	2 µl
Converter Mix E	2 µl	---
Developer Mix J	2 µl	2 µl

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

5. Data Analysis

Correct background by subtracting the value derived from the Sample Background Control from all sample readings. The background reading can be significant and must be subtracted from sample readings. Subtract the zero Inosine Standard from all Standards.

Plot the Inosine Standard Curve. Apply the sample readings to the standard curve to calculate Inosine Concentration (C):

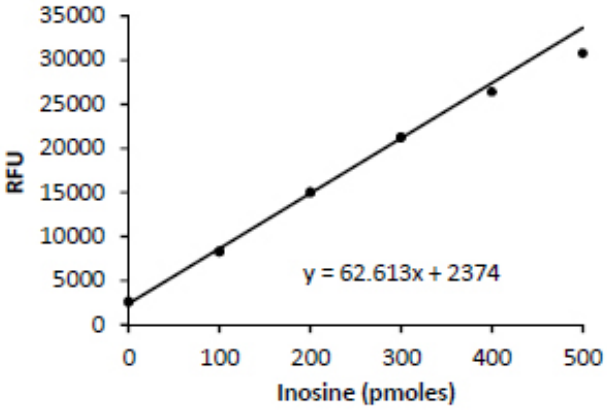
C = Sa / Sv (nmol/µl or mM)

Where:

Sa is the sample amount of unknown (in nmol) from standard curve

Sv is sample volume (µl) added to the wells.

Inosine MW is 268.23 g/mol



6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

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