ab155900

Xanthine/Hypoxanthine Assay Kit

Instructions for Use

For the sensitive and accurate measurement of Xanthine/Hypoxanthine in various tissues/cells and body fluids and the analysis of purine metabolism and cell signaling.

View kit datasheet: www.abcam.com/ab155900

(use www.abcam.cn/ab155900 for China, or www.abcam.co.jp/ab155900 for Japan)

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

Version 5a, Last Updated 5 June 2025

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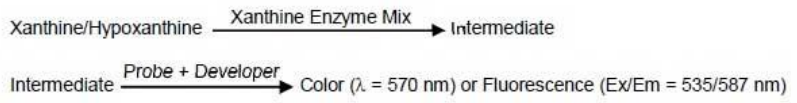
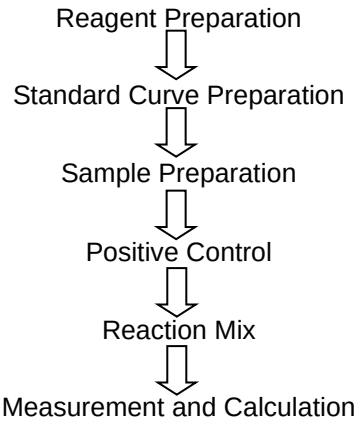
1. Overview

Xanthine, a catabolic product of purine metabolism, is present in body fluids, muscle tissue and certain plants. Structurally like caffeine, Xanthine has a stimulant effect and is used clinically to treat the congestive diseases such as asthma and chronic obstructive pulmonary disease. Xanthine is metabolized into uric acid and superoxide by Xanthine oxidase. Xanthine oxidase deficiency causes the rare genetic disorder-Xanthinuria, and leads to Xanthine accumulation in urine and blood, which ultimately progresses to renal failure. Recent studies show that Xanthine levels are elevated following ischemic injury, thus Xanthine can serve as a useful marker for tissue hypoxia. Early detection of Xanthine alteration in biological fluids is crucial for metabolic studies and for diagnostic and therapeutic monitoring. In Abcam's Xanthine/Hypoxanthine Assay kit, Xanthine/Hypoxanthine is specifically oxidized by the Xanthine Enzyme Mix to form an intermediate, which reacts with Developer Solution V & Probe to form a product that can be measured colorimetrically (λ = 570 nm) or fluorometrically (Ex/Em = 535/587 nm). Xanthine/Hypoxanthine Assay kit is rapid, simple and sensitive. This high-throughput suitable assay kit can detect Xanthine levels as low as 0.4 µM in various biological samples.



2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 2	25 mL
OxiRed™ Probe	0.2 mL
Xanthine Enzyme Mix	1 vial
Developer Solution V	1 vial
Xanthine Standard	1 vial

PLEASE NOTE: Assay Buffer 2 was previously labelled as Assay Buffer II and Assay Buffer. The composition has not changed.

* Store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

B. Additional Materials Required

- 96-well clear plate with flat bottoms (colorimetric)
- 96-well white plate with flat bottoms (fluorometric)
- Multi-well spectrophotometer (ELISA reader)

4. Assay Protocol

A. Reagent Preparation

1. Xanthine Enzyme Mix:

Reconstitute with 220 µl Assay Buffer 2. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.

2. Developer Solution V:

Reconstitute with 220 µl Assay Buffer 2. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.

3. Xanthine Standard:

Reconstitute with 500 µl dH_2O to generate 2.0 mM (2.0 nmol/µl) Xanthine Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.

B. Xanthine Assay Protocol

1. Xanthine Standard Curve:

For colorimetric assay, add 0, 2, 4, 6, 8 & 10 µl of 2 mM Xanthine Standard into series of wells in 96 well plate to generate 0, 4, 8, 12, 16 & 20 nmol/well Xanthine Standard. Adjust volume to 50 µl per well with Assay Buffer 2.

For fluorometric assay, dilute Xanthine Standard to 0.02 mM (20 pmol/µl) by adding 10 µl of 2 mM Xanthine Standard to 990 µl dH_2O & mix. Add 0, 2, 4, 6, 8 & 10 µl of 0.02 mM Xanthine Standard into series of wells in 96 well plate to generate 0, 40, 80, 120, 160 & 200 pmol/well Xanthine Standard. Adjust volume to 50 µl per well with Assay Buffer 2.

2. Sample Preparation:

Liquid samples can be measured directly. Rapidly homogenize tissue (10 mg) or cells (1×10^6) with 100 µl ice cold Assay Buffer 2 for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample per well, adjust final volume to 50 µl with Assay Buffer 2.

Notes:

a) *Some enzymes in samples may interfere with the assay. Enzymes can be removed by 10 K quick spin columns or by perchloric acid/KOH treatment.*
b) *For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.*
c) *For samples having high background, prepare parallel sample well(s) as background control.*

3. Reaction Mix:

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

	Reaction Mix	Background Control Mix
Assay Buffer 2	44 µl	46 µl
Xanthine Enzyme Mix	2 µl	--- µl
Developer Solution V	2 µl	2 µl
Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and test. Mix well.

Note: For samples having high background, add 50 µl of the Background Control Mix to sample background control well(s). Mix well

4. Measurement:

Incubate for 30 min at room temperature, protected from light. Measure fluorescence at Ex/Em = 535/587 nm or color at λ = 570 nm.

5. Data Analysis

Calculation: Subtract 0 Standard reading from all readings. Plot the Xanthine Standard Curve. For samples having high background, correct sample background by subtracting the value derived from the background control from sample readings. Apply the corrected sample reading to the Xanthine Standard Curve to get B pmol or nmol of Xanthine/Hypoxanthine in the sample(s).

$$\text{Xanthine/Hypoxanthine Concentration in sample} = \frac{B}{V} \times \text{Dilution Factor} = \text{nmol/ml/ or pmol/ml = µM or nM}$$

Where:

B is the amount of Xanthine/Hypoxanthine in the sample (pmol or nmol).

V is the sample volume added into the reaction well (ml).

Xanthine molecular weight: 152.11 g/mol. Hypoxanthine molecular weight: 136.11g/mol

Xanthine/Hypoxanthine in samples can also be expressed in nmol/mg of sample or other desired method.

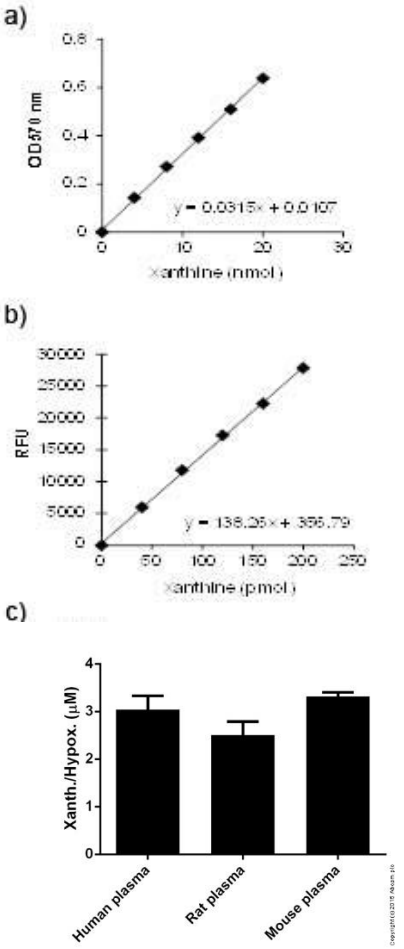


Figure 1: Xanthine Standard Curve (a) & (b). Xanthine & Hypoxanthine measured in biological fluids by fluorometric method showing concentrations (micromolar) (c). Assays were performed following Kit protocol.

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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)