ab102522

Xanthine Oxidase Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Xanthine Oxidase activity in various samples.

View kit datasheet: www.abcam.com/ab102522
(use www.abcam.cn/ab102522 for China, or www.abcam.co.jp/ab102522 for Japan)

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

Version 8a, Last Updated 29 May 2025

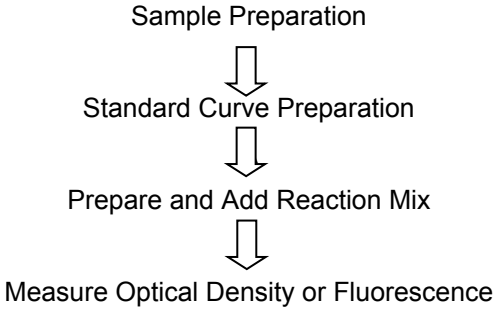
Table of Contents

1. Overview

Xanthine oxidase (XO, EC 1.17.3.2) is present in appreciable amounts in the liver and jejunum in healthy individuals. However, in various liver disorders, XO is released into circulation. Therefore, determination of serum XO level serves as a sensitive indicator of acute liver damage such as jaundice.

Abcam's Xanthine Oxidase Assay Kit is an easy and sensitive assay to determine XO in variety of samples. In the assay, XO oxidizes xanthine to hydrogen peroxide (H_2O_2) which reacts stoichiometrically with OxiRed™ Probe to generate color (at λ = 570 nm) and fluorescence (at Ex/Em = 535/587 nm). Since the color or fluorescence intensity is proportional to XO content, the XO activity can be accurately measured. The kit detects 1-100 mU xanthine oxidase in 100 µl reaction volume.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 7	25 mL
OxiRed™ Probe	0.2 mL
Developer Solution V	1 vial
XO Substrate Mix	1 vial
H_2O_2 Standard	100 µL
Xanthine Enzyme Mix	1 vial

PLEASE NOTE: Developer Solution V was previously labelled as XO Enzyme Mix (Lyophilized), and OxiRed™ Probe as OxiRed™ Probe (in DMSO), and Assay Buffer 7 as Assay Buffer VII and XO Assay Buffer. The composition has not changed.

* Store kit at -20°C, use within 2 months. Read the entire protocol before performing the assay.

OxiRed™ PROBE: Ready to use as supplied. Warm to room temperature to melt frozen DMSO before use. Store at -20°C, protect from light and moisture. Use within 2 months

DEVELOPER SOLUTION V, XO SUBSTRATE MIX,: Dissolve in 220 µl dH_2O. Pipette up and down to dissolve completely

XANTHINE ENZYME MIX: Dissolve in 250 µl dH_2O. Pipette up and down to dissolve completely. Directly before use, dilute by mixing 5 µl Xanthine Enzyme Mix with 45 µl of water to create a positive control working solution.

Or

XO Positive Control (Older lots): Dissolve in 100 µl dH2O. Pipette up and down to dissolve completely.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a. For serum samples: Serum can be directly added into sample wells, and adjust volume to 50 µl/well with dH_2O.

b. For tissue or cell samples: Tissues or cells can be extracted with 4 volumes of the Assay Buffer 7, centrifuge (16,000 x g, 10 min) to get clear XO extract.

Prepare test samples of up to 50 µl/well with Assay Buffer 7 in a 96-well plate.

For the **positive control**, add 5 µl positive control working solution to wells, adjust volume to 50 µl/well with dH_2O.

We suggest testing several doses of your sample to make sure readings are within the standard curve.

Note:

H_2O_2 in the sample will generate background. It is important to set up a background control.

2. Standard Curve Preparation:

Dilute 4 µl of H_2O_2 Standard/0.88 M H_2O_2 Standard into 348 µl dH_2O to generate 10 mM H_2O_2 Standard, then dilute 20 µl of 10 mM H_2O_2 Standard into 980 µl dH_2O to generate 0.2 mM H_2O_2 Standard.

a. **For the colorimetric assay:** Add 0, 10, 20, 30, 40, 50 µl of the 0.2 mM H_2O_2 Standard into 96-well plate in duplicates, bring the total volume to 50 µl each well with dH_2O to generate 0, 2, 4, 6, 8, 10 nmol/well H_2O_2 Standard.

b. **For the fluorometric assay:** Dilute 50 µl fresh 0.2 mM H_2O_2 into 950 µl dH_2O to generate 10 µM H_2O_2 Standard. Add 0, 10, 20, 30, 40, 50 µl of the 10 µM H_2O_2 into 96-well plate in duplicates, bring volume to 50 µl with dH_2O to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well H_2O_2 Standard.

3. Reaction Mix: As H2O2 in the sample will generate background, we recommend to set up a background control for each sample. For standard, positive control and sample wells, use the Sample Reaction Mix. For background control wells for samples, use the Background Control Reaction Mix. Mix enough reagents for the number of assays and standard to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Sample	**Background**

		Control
Assay Buffer 7	44 µl	46 µl
Substrate Mix	2 µl	---
Developer Solution	2 µl	2 µl
V/Enzyme Mix		
OxiRed™ Probe*	2 µl	2 µl

*** Note:** For the fluorescent assay, dilute OxiRed™ probe 10X to reduce background readings.

4. Add 50 µl of the reaction mix to each well containing the H_2O_2 Standard, Positive Control, and test samples, mix well.

5. Measure the plate immediately (OD = 570 nm for colorimetric assay or at Ex/Em = 535/587 nm for fluorometric assay) at T_1 to read A_1, measure again at T_2 after incubating the reaction at 25°C for 10-20 min (or incubate longer time if the sample XO activity is low) to read A_2, protect from light. The signal generated by XO is:

$$\mathbf{\Delta A = A_2 - A_1}$$

Notes:

a) It is essential to read A_1 and A_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A_1 and A_2 in the reaction linear range.

b) Read H_2O_2 standard after 20 min incubation without subtracting A_1. The standard is stable for a few hours.

5. Data Analysis

Subtract background from all readings. For samples, subtract background control reading from sample reading before calculation of ΔA. Plot the H_2O_2 standard Curve. Apply sample ΔA to the H_2O_2 standard curve to get B nmol of H_2O_2 (H_2O_2 generated between T1 and T2 in the reaction by XO).

$$\textbf{XO Activity} = \frac{\textbf{(B x Dilution Factor)}}{\textbf{(}T_2 - T_1\textbf{) x V}} = \textbf{nmol/min/ml = mU/ml}$$

Where:

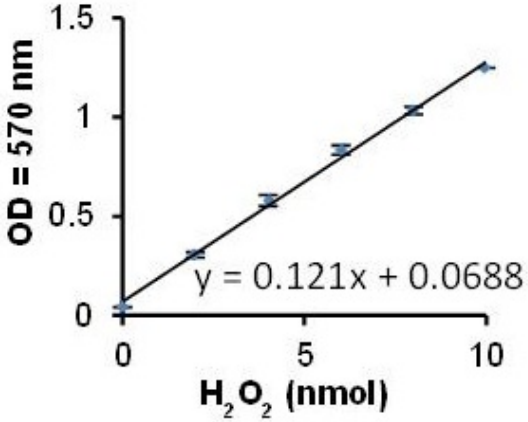
B is the amount of H_2O_2 (nmol) generated by XO from standard curve.

T_1 is the time of the first reading (A_1) (in min).

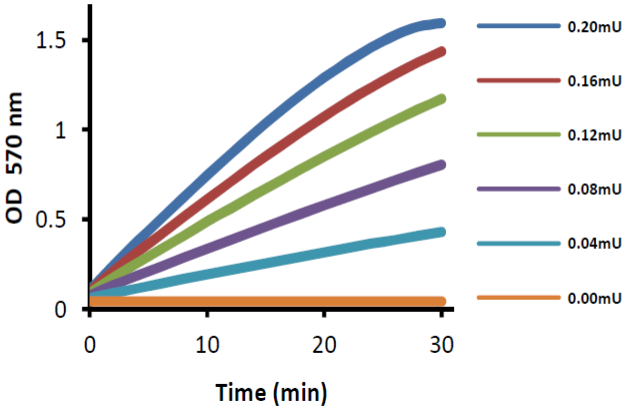
T_2 is the time of the second reading (A_2) (in min).

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

Unit Definition: One unit xanthine oxidase is defined as the amount of enzyme catalyzes the oxidation of xanthine, yielding 1.0 µmol of uric acid and H_2O_2 per minute at 25°C.



Xanthine Oxidase Positive Control



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

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)