Xanthine Oxidase Assay Kit

Catalog Number KA0874

100 assays

Version: 05

Intended for research use only

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Introduction

Background

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. Abnova has developed 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.

General Information

Materials Supplied

List of component

Component	Amount
XO Assay Buffer	25 ml
OxiRed Probe (in DMSO)	200 µl
XO Enzyme Mix (lyophilized)	1 vial
XO Substrate Mix (lyophilized)	1 vial
XO Positive Control	8 µl
H_2O_2 Standard (0.88 M)	0.1 ml

Storage Instruction

This kit is stable for 1 year at -20℃ before opening. After opening, the components should be used within 2 months.

Precautions for Use

For research use only ! Not to be used on humans.

Assay Protocol

Reagent Preparation

- ✓ OxiRed Probe: Ready to use as supplied. (Need to warm up > 20℃ to melt frozen DMSO).
- ✓ XO Enzyme Mix: Dissolve with 220 µl dH_2O. Pipette up and down to dissolve completely.
- ✓ XO Substrate Mix: Dissolve with 220 µl dH_2O. Pipette up and down to dissolve completely.
- ✓ XO Positive Control: Dilute with 92 µl dH_2O. Pipette up and down to dissolve completely.

Assay Procedure

- ✓ Standard Curve Preparations

  Dilute 4 µl of 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.

- 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.
- 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.

- ✓ Sample and Positive Control Preparations

  Prepare test samples in 50 µl/well with assay buffer in a 96-well plate. Serum can be directly added into sample wells, and adjust volume to 50 µl/well with dH_2O. Tissues or cells can be extracted with 4 volume of the Assay Buffer, centrifuge (16,000 x g, 10 min) to get clear XO extract. For the positive control, add 5 µl positive control solution to wells, adjust volume to 50 µl/well with dH_2O. H_2O_2 in the sample will generate background. It is important to set up a background control. We suggest using several doses of your sample to ensure the readings are within the linear range.

- ✓ Reaction Mix Preparation

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

Xanthine oxidase Measurement	Background Control
44 µl Assay Buffer	46 µl Assay Buffer
2 µl Substrate Mix	-
2 µl Enzyme Mix	2 µl Enzyme Mix
2 µl OxiRed Probe**	2 µl OxiRed Probe

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

- ✓ Add 50 µl of the reaction mix to each well containing the H_2O_2 standard, positive controls, and test samples, mix well.

- ✓ Measure the plate immediately (O.D.=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℃ 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 $\Delta A = A_2 - A_1$

  Note:

  1) 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.

  2) Read H_2O_2 standard after 20 min incubation without subtract A1. The standard is stable for a few hours.

Data Analysis

Calculation of Results

Subtract background from all readings. 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 T_1 and T_2 in the reaction by XO).

$$\text{XO Activity} = \frac{B}{(T2 - T1) \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

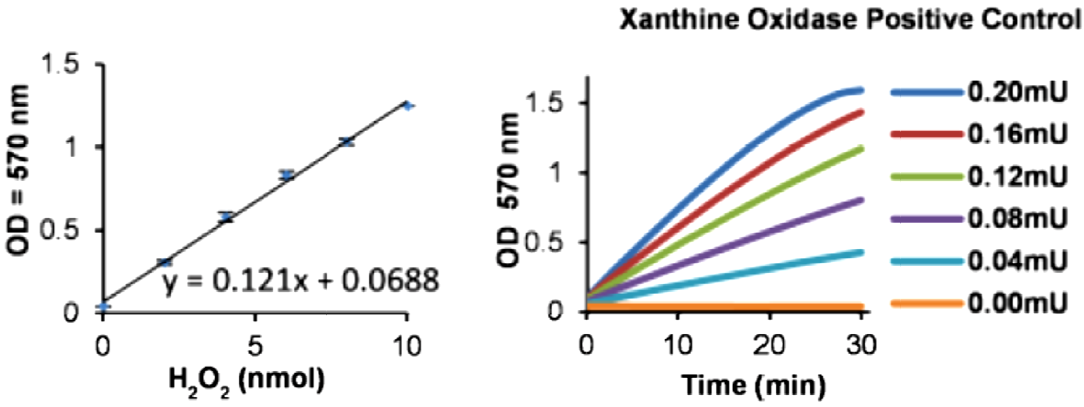
Where: B is the amount of H_2O_2 generated by XO from standard curve (in nmol).

T1 is the time of the first reading (A1) (in min).

T2 is the time of the second reading (A2) (in min).

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

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℃.



Resources

Troubleshooting

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
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • 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 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 • Use fresh samples or store at
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

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
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.		