Technical Bulletin

Xanthine Oxidase Assay Kit

Catalogue number MAK497

Product Description

Xanthine Oxidase catalyzes the oxidation of xanthine to uric acid. In addition, xanthine oxidase can catalyze the oxidation of hypoxanthine to xanthine, act on certain purines and aldehydes, and in certain cases produce the superoxide ion. Clinically, xanthine oxidase activity in blood can act as a marker for influenza, liver damage, and possibly cardiovascular health.

Simple, direct, and high-throughput assays for measuring xanthine oxidase activity find wide applications in research and drug discovery. The Xanthine Oxidase Assay Kit uses a single working reagent that combines the xanthine oxidase reaction and the color reaction in one step. The change in color intensity of the reaction product at 570 nm or fluorescence intensity at λ_{Ex} =530 nm/λ_{Em} =585 nm is directly proportional to xanthine oxidase activity in the sample.

The linear detection range of the kit is 0.03 to 25 U/L xanthine oxidase for colorimetric assays and 0.01 to 2.5 U/L for fluorometric assays. The kit is suitable for xanthine oxidase activity determination in cell lysate, serum, and other biological samples, as well as for studying the effects of drugs on xanthine oxidase metabolism.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK497A
- HRP Enzyme 120 µL
  Catalogue Number MAK497B
- 5 mM Xanthine 1.5 mL
  Catalogue Number MAK497C
- Dye Reagent 120 µL
  Catalogue Number MAK497D
- Standard (3% H_2O_2) 100 µL
  Catalogue Number MAK497E

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

HRP Enzyme: During experiment, keep HRP Enzyme in a refrigerator or on ice.

Colorimetric Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

1. Samples can be analyzed immediately after collection, or stored in aliquots at –20 °C.
2. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge Sample and use clear supernatant for assay.
3. Transfer 10 µL of each Sample into separate wells of a clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay.

Colorimetric Standard Curve Preparation:

1. Prepare a 4.8 mM H_2O_2 Standard solution by mixing 5 µL 3% H_2O_2 and 914 µL purified water.
2. Further, prepare a 400 µM Standard solution by mixing 20 µL of the 4.8 mM H_2O_2 solution with 220 µL purified water.
3. Use 400 µM Standard solution to prepare Standards shown in Table 1

Table 1.
Preparation of H_2O_2 Colorimetric Standards

Well	400 µM Standard	Purified Water	H_2O_2 (µM)
1	100 µL	0 µL	400
2	60 µL	40 µL	240
3	30 µL	70 µL	120
4	0 µL	100 µL	0

4. Mix well and transfer 10 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Dilute the Standards #1, #2, and #3 from Colorimetric Standard Curve Preparation 10X with purified water as shown in Table 2.

Table 2.
Preparation of H_2O_2 Fluorometric Standards

Well	Standards (from Table 1)		Purified Water	H_2O_2 (µM)
1	Standard #1	10 µL	90 µL	40
2	Standard #2	10 µL	90 µL	24
3	Standard #3	10 µL	90 µL	12
4	N/A	0 µL	100 µL	0

2. Mix well and transfer 10 µL of each Standard into separate wells of a black 96 well plate.

Working Reagent Preparation

Mix enough reagent for the number of assays to be performed. For each Standard and Sample well, prepare 97 µL of Working Reagent according to Table 3.

Table 3.
Preparation of Working Reagents

Reagent	Working Reagent
Assay Buffer	85 µL
5 mM Xanthine	10 µL
HRP Enzyme	1 µL
Dye reagent	1 µL

Transfer 90 µL Working Reagent into each reaction (and control) well. Tap plate to mix.

Measurement

1. Read optical density (OD_o) at 570 nm (550-585 nm) or fluorescence intensity (F_0) at λ_{Ex} =530 nm/λ_{Em} =585 nm immediately
2. Incubate 20 min at room temperature, and then read optical density (OD_{20}) at 570 nm (550-585 nm) or fluorescent intensity (F_{20}) at λ_{Ex} =530 nm/λ_{Em} =585 nm again.

Results

1. Subtract blank OD_{20} or F_{20} (water, #4) from all standard OD_{20} or F_{20} values.
2. Plot the ΔF or ΔOD against the standard concentrations.
3. Determine the slope using linear regression. Calculate the ΔOD_{Sample} or ΔF_{Sample} of all Samples by subtracting OD_0 or F_0 from OD_{20} or F_{20} for each sample. Do the same for the blank (water, Standard #4) to get ΔOD_{Blank} or ΔF_{Blank}

Calculate the Xanthine Oxidase activity using the equation below:

Xanthine Oxidase (U/L) =

$$\frac{\Delta R_{Sample} - \Delta R_{Blank}}{\text{Slope}\left(\mu M^{-1}\right)\cdot t} \times DF$$

Where:
ΔR_{Sample} = the change in optical density or fluorescent values of the sample
ΔR_{Blank} = the change in optical density or fluorescent values of the blank
Slope = the slope of the H_2O_2 standard curve.
t = the incubation time (20 minutes)
DF = the dilution factor.
Unit Definition: U/L of Xanthine Oxidase catalyzes the conversion of 1 µMole of Xanthine to uric acid per minute at pH 7.0 and room temperature.

Note: If the calculated Sample XO activity is higher than 25 U/L in colorimetric assay or 2.5 U/L in fluorometric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor (DF). For samples with low Xanthine Oxidase activity, the incubation time can be increased to up to 2 hours.

Figure 1.
Typical Colorimetric Xanthine Oxidase Standard Curve

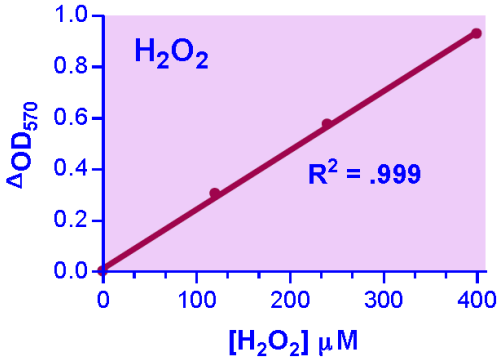
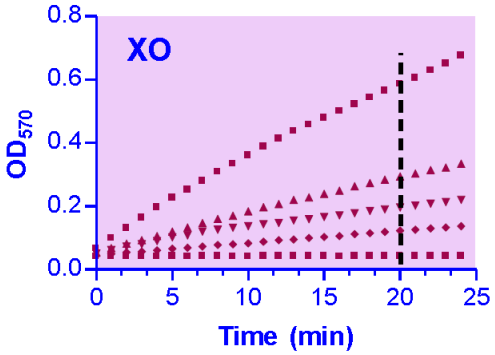


Figure 2.
Typical Colorimetric Xanthine Oxidase OD_{570} Readings



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