

EnzyChrom™ Xanthine Oxidase Assay Kit (EXOX-100)

Quantitative Colorimetric/Fluorimetric Xanthine Oxidase Activity Determination

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. BioAssay Systems' xanthine oxidase assay kit uses a single Working Reagent that combines the xanthine oxidase reaction and color reaction in one step. The change in color intensity of the reaction product at 570 nm or fluorescence intensity at $\lambda_{ex/em} = 530/585$ nm is directly proportional to xanthine oxidase activity in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 10 μ L samples. Linear detection range in 96-well plate for 20 minute incubation: 0.03 to 25 U/L xanthine oxidase for colorimetric assays and 0.01 to 2.5 U/L for fluorimetric assays.

Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 20 min at room temperature.

APPLICATIONS

Direct Assays: xanthine oxidase activity in cell lysate, serum, and other biological samples.

Drug Discovery/Pharmacology: effects of drugs on xanthine oxidase metabolism.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 10 mL	HRP Enzyme: 120 μ L
Xanthine: 1.5 mL 5 mM Xanthine	Dye Reagent: 120 μ L
Standard: 100 μ L 3% H ₂ O ₂	

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIMETRIC PROCEDURE

Samples can be analyzed immediately after collection, or stored in aliquots at -20°C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

- Equilibrate all components to room temperature. During experiment, keep thawed Enzyme in a refrigerator or on ice.
- H₂O₂ Standard Curve.** Mix 5 μ L 3% H₂O₂ and 914 μ L dH₂O (final 4.8 mM) then mix 20 μ L of the 4.8 mM H₂O₂ with 220 μ L dH₂O to yield 400 μ M H₂O₂. Prepare standards as shown in the Table below.

No	400 μ M H ₂ O ₂ + H ₂ O	Vol (μ L)	H ₂ O ₂ (μ M)
1	100 μ L + 0 μ L	100	400
2	60 μ L + 40 μ L	100	240
3	30 μ L + 70 μ L	100	120
4	0 μ L + 100 μ L	100	0

Transfer 10 μ L standards and samples into separate wells.

- Working Reagent.** Prepare bulk working reagent by mixing 85 μ L Assay Buffer, 10 μ L 5 mM Xanthine, 1 μ L HRP Enzyme (*vortex briefly before pipetting*), and 1 μ L Dye Reagent per reaction well in a clean tube. Transfer 90 μ L Working Reagent into each reaction well. Tap plate to mix.
- Read optical density immediately (OD₀) at 570 nm (550-585 nm). Incubate 20 min at room temperature, and then read optical density again (OD₂₀).

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 0.01 to 2.5 U/L xanthine oxidase. Dilute the standards from *Colorimetric Procedure* 10 \times with dH₂O to obtain standards at 40, 24, 12 and 0 μ M H₂O₂.

Transfer 10 μ L standards and 10 μ L samples into separate wells of a *black* 96-well plate.

Add 90 μ L Working Reagent (see *Colorimetric Procedure*), tap plate to mix.

Read fluorescence immediately (F₀) at $\lambda_{ex/em} = 530/585$ nm, incubate 20 min at room temperature, and then read fluorescence again (F₂₀).

CALCULATION

Subtract blank OD₂₀ or F₂₀ (water, #4) from all standard OD₂₀ or F₂₀ values and plot the Δ OD or Δ F against standard concentrations. Determine the slope using linear regression. Calculate the Δ OD_{Sample} or Δ F_{Sample} of all samples by subtracting OD₀ or F₀ from OD₂₀ or F₂₀ for each sample. Do the same for the blank (water, standard #4) to get Δ OD_{Blank} or Δ F_{Blank}. Calculate the activity using the equation below:

$$\text{XO Activity} = \frac{\Delta R_{\text{SAMPLE}} - \Delta R_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1}) \cdot t} \times n \quad (\text{U/L})$$

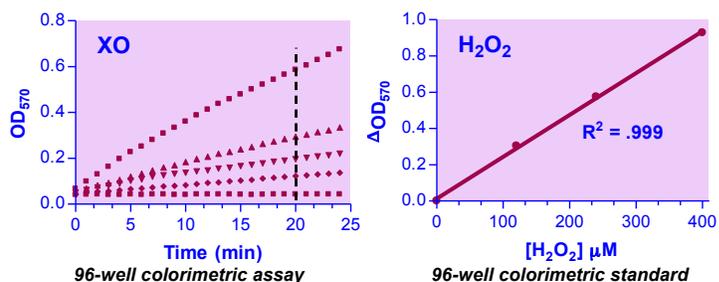
Where ΔR_{Sample} and ΔR_{Blank} are the change in optical density or fluorescent values of the sample and blank, respectively. *Slope* is the slope of the H₂O₂ standard curve, *t* is the incubation time (20 minutes), and *n* is the dilution factor.

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

Unit definition: 1 U/L of Xanthine Oxidase catalyzes the conversion of 1 μ mole of Xanthine to uric acid per minute at pH 7.0 and room temperature.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), black 96-well plates (e.g. Greiner Bio-One, cat# 655900) and plate reader.



LITERATURE

- Enroth C, Eger BT, Okamoto K. (2000). Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion. *Proc. Natl. Acad. Sci. USA* 97(20):10723-8.
- Harrison R. (2002). Structure and function of xanthine oxidase: where are we now? *Free Radic. Biol. Med.* 33(6):774-97.
- Higgins P, Dawson J, Walters M. (2009). The Potential for Xanthine Oxidase Inhibition in the Prevention and Treatment of Cardiovascular and Cerebrovascular Disease. *Cardiovascular Psychiatry and Neurology* 2009: 1-9.