# Technical Manual

## ColorFluor Xanthine Oxidase Activity Assay Kit

## Catalogue Code: BA0155

## Pack Size: 100 assays

## Research Use Only

## 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 Assay Genie ColorFluor Xanthine Oxidase Activity 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 µL 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 µL |
| **Xanthine:** | 1.5 mL 5 mM Xanthine | **Dye Reagent:** | 120 µL |
| **Standard:** | 100 µL 3% $H_2O_2$ | | |

**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.

1. Equilibrate all components to room temperature. During experiment, keep thawed Enzyme in a refrigerator or on ice.
2. *$H_2O_2$ Standard Curve*. Mix 5 µL 3% $H_2O_2$ and 914 µL $dH_2O$ (final 4.8 mM) then mix 20 µL of the 4.8 mM $H_2O_2$ with 220 µL $dH_2O$ to yield 400 µM $H_2O_2$. Prepare standards as shown in the Table below.

| No | 400 µM $H_2O_2$ + $H_2O$ | Vol (µL) | $H_2O_2$ (µM) |
|---|---|---|---|
| 1 | 100 µL + 0 µL | 100 | 400 |
| 2 | 60 µL + 40 µL | 100 | 240 |
| 3 | 30 µL + 70 µL | 100 | 120 |
| 4 | 0 µL + 100 µL | 100 | 0 |

Transfer 10 µL standards and samples into separate wells.

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

**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× with $dH_2O$ to obtain standards at 40, 24, 12 and 0 µM $H_2O_2$.

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

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

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

**CALCULATION**

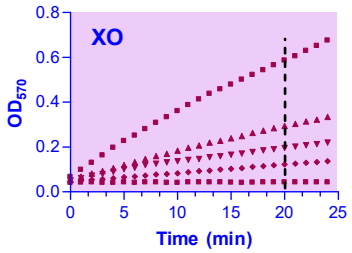
Subtract blank $OD_{20}$ or $F_{20}$ (water, #4) from all standard $OD_{20}$ or $F_{20}$ values and plot the ΔOD or ΔF against standard concentrations. Determine the slope using linear regression. Calculate the $\Delta OD_{Sample}$ or $\Delta F_{Sample}$ of all samples by subtracting $OD_0$ or $F_0$ from $OD_{20}$ or $F_{20}$ for each sample. Do the same for the blank (water, standard #4) to get $\Delta OD_{Blank}$ or $\Delta F_{Blank}$. Calculate the activity using the equation below:

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

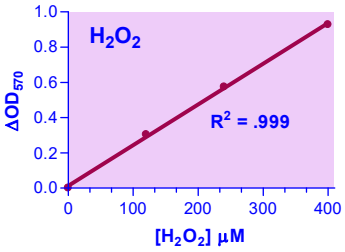
Where $\Delta R_{Sample}$ and $\Delta R_{Blank}$ are the change in optical density or fluorescent values of the sample and blank, respectively. *Slope* is the slope of the $H_2O_2$ standard curve, *t* is the incubation time (20 minutes), and *n* is the dilution factor.

***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 µmole of Xanthine to uric acid per minute at pH 7.0 and room temperature.




*96-well colorimetric assay*




*96-well colorimetric standard*

**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**

1. 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.
2. Harrison R. (2002). Structure and function of xanthine oxidase: where are we now? Free Radic. Biol. Med. 33(6):774-97.
3. 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.

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