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Product Information

Xanthine Oxidase Activity Assay Kit

Catalog Number **MAK078** Storage Temperature –20 °C

TECHNICAL BULLETIN

25 ml

0.1 mL

Product Description

Xanthine Oxidase (XO) catalyzes the sequential oxidation of hypoxanthine to xanthine, and xanthine to uric acid and hydrogen peroxide. In humans and other primates, XO controls the final step of purine catabolism and is normally found in the liver and the intestinal mucosa. In rodents, XO is broadly expressed in most tissues. While XO activity is normally very low in blood, liver injury can result in the release of XO into blood. XO may contribute to the pathogenesis of gout and cardiovascular disease, and XO activity or expression may be upregulated in these conditions.

The Xanthine Oxidase Activity Assay Kit provides a simple and direct procedure for measuring XO activity in a variety of biological samples. XO activity is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product, proportional to the hydrogen peroxide generated. One unit of XO is defined as the amount of enzyme that catalyzes the oxidation of xanthine, yielding 1.0 µmole of uric acid and hydrogen peroxide per minute at 25 °C.

Components

Vanthina Ovidaga Assay Buffor

Hydrogen Peroxide Standard, 0.88 M

Catalog Number MAK078F

The kit is sufficient for 100 assays in 96 well plates.

Catalog Number MAK078A	25 ML
Fluorescent Peroxidase Substrate, in DMSO Catalog Number MAK078B	0.2 mL
Xanthine Oxidase Enzyme Mix Catalog Number MAK078C	1 vl
Xanthine Oxidase Substrate Mix Catalog Number MAK078D	1 vl
Xanthine Oxidase Positive Control Catalog Number MAK078E	8 μL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Xanthine Oxidase Assay Buffer – Allow buffer to come to room temperature before use.

Fluorescent Peroxidase Substrate – Allow reagent to come to room temperature before use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C. Upon thawing, the Fluorescent Peroxidase Substrate is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Fluorescent Peroxidase Substrate 5 to 10-fold with Xanthine Oxidase Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Xanthine Oxidase Substrate Mix and Xanthine Oxidase Enzyme Mix – Reconstitute each in 220 μL of water. Mix well by pipetting, then aliquot and store at –20 °C. Use within two months of reconstitution.

Xanthine Oxidase Positive Control – Dilute with 92 μ L of water. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use. Use within two months of reconstitution.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

<u>Hydrogen Peroxide Standards for Colorimetric</u> Detection

Dilute 4 μ L of the 0.88 M Hydrogen Peroxide Standard with 348 μ l of water to prepare a 10 mM standard solution. Dilute 20 μ L of the 10 mM standard with 980 μ L of water to generate a 0.2 mM Hydrogen Peroxide Standard. Add 0, 10, 20, 30, 40, and 50 μ L of the 0.2 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add water to each well to bring the volume to 50 μ L.

<u>Hydrogen Peroxide Standards for Fluorometric Detection</u>

Prepare a 0.2 mM standard solution as for the colorimetric assay. Dilute 50 μ L of the 0.2 mM standard solution with 950 μ L of water to make a 10 μ M standard solution. Add 0, 10, 20, 30, 40, and 50 μ L of the diluted 10 μ M standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add water to each well to bring the volume to 50 μ L.

Sample Preparation

Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Tissue or cells should be rapidly homogenized with 4 volumes of Xanthine Oxidase Assay Buffer. Centrifuge at $15,000 \times g$ for 10 minutes to remove insoluble materials. Bring samples to a final volume of $50~\mu L$ with Xanthine Oxidase Assay Buffer.

Serum samples can be directly added to wells. Add 1–50 μ L samples into wells of a 96 well plate. Bring samples to final volume of 50 μ L with Xanthine Oxidase Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

For the positive control (optional), add 5 μ L of the Xanthine Oxidase Positive Control to wells. Adjust well volume to 50 μ L with water.

Hydrogen Peroxide in the samples will generate a background signal. To remove the effect of hydrogen peroxide background, a sample blank may be set up by omitting the Xanthine Oxidase Substrate Mix. The blank readings can then be subtracted from the sample readings.

Assay Reaction

 Set up the Reaction Mixes according to the scheme in Table 1. Prepare enough Reaction Mixes for the number of samples, positive controls, and standards to be performed.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Xanthine Oxidase Assay Buffer	44 μL	46 μL
Xanthine Oxidase Substrate Mix	2 μL	_
Xanthine Oxidase Enzyme Mix	2 μL	2 μL
Fluorescent Peroxidase Substrate	2 μL	2 μL

- 2. Add 50 μ L of the appropriate Reaction Mix to each well. Mix well using a horizontal shaker or by pipetting.
- 3. After 2–3 minutes, take the initial measurement ($T_{initial}$). For colorimetric assays, measure the absorbance at 570 nm (A_{570})_{initial}. For fluorometric assays, measure fluorescence intensity (FLU_{initial}, $\lambda_{ex} = 535/\lambda_{em} = 587$ nm).
- 4. Incubate the plate at 25 °C taking measurements every 5 minutes. Protect the plate from light during the incubation.

- Continue taking measurements until the value of the most active sample is greater than the value of the highest standard. At this time the most active sample is near or exceeds the end of the linear range of the standard curve.
- The final measurement for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve. The time of the penultimate reading is T_{final}.
- 7. Calculate the change in measurement from T_{initial} to T_{final} .

$$\Delta A_{570} = (A_{570})_{\text{final}} - (A_{570})_{\text{initial}}$$

or

$$\Delta FLU = (FLU_{final}) - (FLU_{initial})$$

<u>Note</u>: It is essential the initial and final measurements fall within the linear range of the reaction.

Results

Calculations

Correct for the background by subtracting the value obtained for the 0 (blank) standard from all readings. Plot the hydrogen peroxide standard curve.

Compare the Δ measurement value (ΔA_{570} or Δ FLU) of each sample to the standard curve to determine the amount of hydrogen peroxide generated (B) between T_{initial} and T_{final} .

Note: A new standard curve must be set up each time the assay is run.

The XO activity of a sample may be determined by the following equation:

XO Activity =
$$\underline{B \times Sample\ Dilution\ Factor}$$

 $(T_{final} - T_{initial}) \times V$

B = Amount (nmole) of hydrogen peroxide generated between $T_{initial}$ and T_{final}

 T_{initial} = Time of first reading in minutes. T_{final} = Time of second reading in minutes. V = sample volume (mL) added to well.

XO activity is reported as nmole/min/mL = milliunit/mL, where one milliunit (mU) of XO is defined as the amount of enzyme that catalyzes the oxidation of xanthine yielding 1.0 μ mole of uric acid and hydrogen peroxide per minute at 25 °C.

Troubleshooting Guide

Assay Not Working Cold assay buffer	Problem		Suggested Solution	
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