

Xanthine Oxidase (XOD) Activity Assay Kit - Micro Method**Product Information**

Product Code	55446
Assay Size	100T
Detection Wavelength	290 nm

Product Introduction

Xanthine oxidase (XOD, EC 1.17.3.2) is mainly distributed in mammalian tissues such as the heart, lungs, and liver. When liver function is impaired, XOD is released in large amounts into serum, making it significant for the diagnosis of liver damage.

XOD catalyzes the oxidation of xanthine to uric acid and superoxide anions. It is one of the main sources of reactive oxygen species and is also a key enzyme in nucleotide metabolism.

Uric acid has a characteristic absorption peak at 290 nm. By measuring the change in absorbance at 290 nm, the rate of uric acid production can be detected and used to calculate XOD enzyme activity.

Sample used for the measured effect diagram: xanthine oxidase preparation. Under different assay conditions, actual readings may vary depending on the detection instrument. The data are for reference only.

Package List and Storage

Code	Component	Volume / Quantity	Storage
55446.1	Extraction Solution	110 mL	2-8 °C
55446.2	Reagent I	25 mL	2-8 °C
55446.3	Reagent II	1.9 mg x 2	2-8 °C, protected from light
55446.m	Instruction Manual	1 copy	-

Quality Standards and Safety Information

Raw Material or Package Name	Quality Standard	Main Toxicity
Extraction Solution	-	-
Reagent I	-	-
Reagent II	-	-

Transportation and Storage

This product is shipped with ice packs.

Store the product according to the storage conditions in this manual. Shelf life: 180 days.

Instructions for Use**1. Preparation of Crude Enzyme Extract****Bacterial, Cell, or Tissue Samples**

For bacteria or cultured cells, collect the bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant.

Add Extraction Solution according to a bacterial or cell count of $500\text{-}1000 \times 10^4$ cells per 1 mL Extraction Solution. The recommended ratio is 500×10^4 bacteria or cells with 1 mL Extraction Solution.

Disrupt the bacteria or cells by ultrasonication in an ice bath using 20% power or 200 W. Sonicate for 3 s, pause for 10 s, and repeat 30 times.

Centrifuge at 8000g, 4 °C for 10 min. Collect the supernatant and keep it on ice for testing.

For tissue samples, add Extraction Solution according to a tissue mass (g) to Extraction Solution volume (mL) ratio of 1:5-10. The recommended preparation is approximately 0.1 g tissue with 1 mL Extraction Solution.

Homogenize in an ice bath, then centrifuge at 8000g, 4 °C for 10 min. Collect the supernatant and keep it on ice for testing.

Serum, Plasma, and Other Liquid Samples

Serum, plasma, and other liquid samples can be tested directly. If precipitate is present, centrifuge before measurement.

2. Operating Procedure

1. Preheat the UV spectrophotometer or microplate reader for more than 30 min. Set the wavelength to 290 nm and zero with distilled water.
2. Prepare the XOD detection working solution before use. Add 11 mL Reagent I to each bottle of Reagent II, mix thoroughly, and set aside for use. Unused solution can be stored at 4 °C for one week.
3. Before measurement, incubate the XOD assay working solution in a 37 °C water bath for mammals or a 25 °C water bath for other species for 10 min or longer.
4. Add 10 μL sample and 200 μL working solution to a micro quartz cuvette or 96-well UV plate. Immediately mix and start timing.
5. Record the initial absorbance at 290 nm as A1. Record the absorbance after 1 min as A2.
6. Calculate $\Delta A = A2 - A1$.

XOD Activity Calculation

A. Calculation Using a Micro Quartz Cuvette

Serum or Plasma

Unit definition: one unit of enzyme activity is defined as the amount that catalyzes the production of 1 nmol uric acid per milliliter of serum or plasma per minute.

$$\text{XOD (U/mL)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div V_{\text{sample}} \div T = 1721.3 \times \Delta A$$

Calculation by Sample Protein Concentration

Unit definition: the amount that catalyzes the production of 1 nmol uric acid per mg of tissue protein per minute is defined as one unit of enzyme activity.

$$\text{XOD (U/mg prot)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (V_{\text{sample}} \times C_{\text{pr}}) \div T = 1721.3 \times \Delta A \div C_{\text{pr}}$$

Calculation by Sample Fresh Weight

Unit definition: the amount that catalyzes the production of 1 nmol uric acid per g of tissue per minute is defined as one unit of enzyme activity.

$$\text{XOD (U/g mass)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 1721.3 \times \Delta A \div W$$

Calculation by Bacterial or Cell Density

Unit definition: the amount that catalyzes the production of 1 nmol uric acid per 10^4 bacteria or cells is defined as one unit of enzyme activity.

$$\text{XOD (U/}10^4\text{cell)} = [\Delta A \times V_{\text{reaction total}} \div (\epsilon \times d) \times 10^9] \div (N \times V_{\text{sample}} \div V_{\text{sample total}}) \div T = 1721.3 \times \Delta A \div N$$

B. Calculation Using a 96-Well UV Plate

Serum or Plasma

Unit definition: one unit of enzyme activity is defined as the amount that catalyzes the production of 1 nmol uric acid per milliliter of serum or plasma per minute.

$$\text{XOD (U/mL)} = [\Delta A \times V_{\text{reaction total}} \div (\epsilon \times d) \times 10^9] \div V_{\text{sample}} \div T = 3442.6 \times \Delta A$$

Calculation by Sample Protein Concentration

Unit definition: the amount that catalyzes the production of 1 nmol uric acid per mg of tissue protein per minute is defined as one unit of enzyme activity.

$$\text{XOD (U/mg prot)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (V_{\text{sample}} \times \text{Cpr}) \div T = 3442.6 \times \Delta A \div \text{Cpr}$$

Calculation by Sample Fresh Weight

Unit definition: the amount that catalyzes the production of 1 nmol uric acid per g of tissue per minute is defined as one unit of enzyme activity.

$$\text{XOD (U/g mass)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 3442.6 \times \Delta A \div W$$

Calculation by Bacterial or Cell Density

Unit definition: the amount that catalyzes the production of 1 nmol uric acid per 10^4 bacteria or cells per minute is defined as one unit of enzyme activity.

$$\text{XOD (U/}10^4\text{cells)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (N \times V_{\text{sample}} \div V_{\text{sample total}}) \div T = 3442.6 \times \Delta A \div N$$

Formula Parameters

Parameter	Description
$V_{\text{reaction total}}$	Total volume of the reaction system: 2.1×10^{-4} L
ϵ	Molar extinction coefficient of uric acid: 1.22×10^4 L/mol/cm
d	Optical path length: 1 cm for a micro quartz cuvette; 0.5 cm for a 96-well UV plate
10^9	Unit conversion factor: $1 \text{ mol} = 10^9 \text{ nmol}$
V_{sample}	Volume of sample added: 0.01 mL
$V_{\text{sample total}}$	Volume of Extraction Solution added: 1 mL
T	Reaction time: 1 min
W	Sample mass, g
Cpr	Sample protein concentration, mg/mL
N	Total number of cells or bacteria

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for preliminary testing. This 100T kit can test 96 samples.
2. Required instruments and supplies prepared by the user include a UV spectrophotometer or microplate reader, benchtop centrifuge, adjustable pipette, water bath or constant-temperature incubator, micro quartz cuvette or 96-well UV plate, homogenizer or mortar, cell ultrasonic disruptor, ice, and distilled water.

Visual Reference