

## Xanthine Oxidase (XOD) Activity Assay Kit

Product code: 55821

Method: Spectrophotometric method

## 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, which is significant for the diagnosis of liver damage.

XOD catalyzes the oxidation of xanthine to produce uric acid and superoxide anion. It is one of the main sources of reactive oxygen species and one of the key enzymes 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 formation can be detected, representing XOD enzyme activity.

Sample in validation data: xanthine oxidase preparation. Actual readings may vary depending on assay conditions and the instrument used. Data are for reference only.

## Package Contents

Item	Component	Amount	Storage
55821.1	Extraction Solution	60 mL	2-8°C
55821.2	Reagent I	55 mL	2-8°C
55821.3	Reagent II	4.5 mg x 2	2-8°C, protected from light
55821.m	Manual	1 copy	/

## Quality and Safety Information

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

## Transportation and Storage

Transportation: This product is transported with ice packs.

Storage: Store each component according to the conditions listed in this manual. Shelf life is 180 days.

## Instructions for Use

### 1. Crude Enzyme Extract Preparation

#### 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 the ratio of bacteria or cells to Extraction Solution volume: 5,000,000-10,000,000 cells per 1 mL. The recommended ratio is 5,000,000 bacteria or cells plus 1 mL Extraction Solution.

Disrupt the bacteria or cells by ultrasonication in an ice bath at 20% power or 200 W. Sonicate for 3 s, pause for 10 s, and repeat 30 times. Centrifuge at 8000 x g, 4°C for 10 min. Collect the supernatant and keep it on ice for testing.

For tissue samples, add Extraction Solution according to the ratio of tissue mass to Extraction Solution volume: 1 g tissue to 5-10 mL Extraction Solution. The recommended amount is approximately 0.1 g tissue plus 1 mL Extraction Solution.

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

## **Serum, Plasma, and Other Liquid Samples**

Test serum, plasma, and other liquid samples directly. If precipitate is present, centrifuge before measurement.

## **2. Operating Procedure**

1. Preheat the spectrophotometer for at least 30 min. Set the wavelength to 290 nm and zero the instrument with distilled water.
2. Prepare the XOD detection working solution before use. Add 26 mL Reagent I to each bottle of Reagent II, mix thoroughly, and set aside for use. Unused working solution can be stored at 4°C for one week.
3. Before measurement, place the XOD detection working solution in a water bath at 37°C for mammals or 25°C for other species for at least 10 min.
4. In a 1 mL quartz cuvette, add 35  $\mu$ L sample and 1 mL working solution. Mix immediately 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**

### **1. Serum or Plasma XOD Calculation**

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

$$\text{XOD (U/mL)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div V_{\text{sample}} \div T = 2423.9 \times \Delta A$$

### **2. Tissue, Bacteria, or Cell XOD Calculation**

#### **Calculated by Sample Protein Concentration**

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

$$\text{XOD (U/mg prot)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (V_{\text{sample}} \times C_{\text{pr}}) \div T = 2423.9 \times \Delta A \div C_{\text{pr}}$$

#### **Calculated by Fresh Sample Weight**

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

$$\text{XOD (U/g mass)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 2423.9 \times \Delta A \div W$$

#### **Calculated by Bacterial or Cell Density**

Unit definition: The amount of enzyme in every 10<sup>4</sup> bacteria or cells that catalyzes the production of 1 nmol uric acid is defined as one unit of enzyme activity.

$$\text{XOD (U/10}^4\text{cell)} = [\Delta A \times V_{\text{total reaction}} \div (\epsilon \times d) \times 10^9] \div (N \times V_{\text{sample}} \div V_{\text{total sample}}) \div T = 2423.9 \times \Delta A \div N$$

## Formula Parameters

Parameter	Meaning	Value
V <sub>total reaction</sub>	Total volume of the reaction system	1.035 x 10 <sup>-3</sup> L
ε	Molar extinction coefficient of uric acid	1.22 x 10 <sup>4</sup> L/mol/cm
d	Optical path length of the 1 mL quartz cuvette	1 cm
10 <sup>9</sup>	Unit conversion factor	1 mol = 10 <sup>9</sup> nmol
V <sub>sample</sub>	Volume of sample added	0.035 mL
V <sub>total sample</sub>	Volume of extract added	1 mL
T	Reaction time	1 min
W	Sample mass	g
C <sub>pr</sub>	Sample protein concentration	mg/mL
N	Total number of cells or bacteria	--

## Precautions

1. Before formal determination, select 2-3 samples with large expected differences for preliminary testing.
2. This 50T kit can test 48 samples.
3. Materials and instruments required but not provided: UV spectrophotometer, benchtop centrifuge, adjustable pipette, water bath or constant-temperature incubator, 1 mL quartz cuvette, homogenizer or mortar, ultrasonic cell disruptor, ice, and distilled water.

## Visual Reference