Xanthine Oxidase (XOD) Activity Assay Kit - Microplate Method

Product code: 55572

Product Introduction

Xanthine oxidase (XOD, EC 1.17.3.2) is mainly distributed in mammalian heart, lung, liver, and other tissues. When liver function is impaired, XOD is released into serum in large amounts, which has specific significance for the diagnosis of liver damage.

XOD catalyzes the oxidation of xanthine to generate uric acid and superoxide anions. 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 generation can be detected and used to represent 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. Reference data are for guidance only.

Package Contents

Code	Component	Quantity	Storage
55572.1	Extraction Solution	110 mL	2-8°C
55572.2	Reagent I	25 mL	2-8°C
55572.3	Reagent II	1.9 mg x 2	2-8°C, protected from light
55572.m	Instruction Manual	1 copy	-

Quality and Safety Information

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

Transportation and Storage

Transportation: This product is transported with ice packs.

Storage: Store according to this instruction manual. Shelf life is 180 days.

Assay Procedure

1. Preparation of Crude Enzyme Extract

Bacterial, Cell, or Tissue Samples

For bacteria or cultured cells, collect the bacteria or cells in a centrifuge tube, centrifuge, and discard the supernatant. Add Extraction Solution according to the ratio of bacteria or cells (10^4cells) to Extraction Solution volume (mL) of 500-1000:1. It is recommended to use 1 mL Extraction Solution for 5,000,000 bacteria or cells.

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

For tissue samples, add Extraction Solution according to the ratio of tissue mass (g) to Extraction Solution volume (mL) of 1:5-10. It is recommended to weigh approximately 0.1 g tissue and add 1 mL Extraction Solution. Homogenize in an ice bath. Centrifuge at 8000g and 4°C for 10 minutes. 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 the sample before measurement.

2. Operating Steps

1. Preheat the microplate reader for more than 30 minutes. Set the wavelength to 290 nm and zero the instrument with distilled water.
2. Prepare the XOD detection working solution before use. Add 11 mL Reagent I to each bottle of Reagent II and mix thoroughly. Unused working solution can be stored at 4°C for one week.
3. Before measurement, place the XOD detection working solution in a 37°C water bath for mammals or a 25°C water bath for other species for at least 10 minutes.
4. Add 10 μL sample and 200 μL working solution to a 96-well UV plate. Mix immediately and start timing.
5. Record the initial absorbance at 290 nm as A1. Record the absorbance after 1 minute as A2.
6. Calculate ΔA = A2 - A1.

XOD Activity Calculation

Serum or Plasma Samples

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

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

Tissue, Bacterial, or Cell Samples

Calculated by sample protein concentration:

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

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

Calculated by sample fresh weight:

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

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

Calculated by bacterial or cell density:

Unit definition: One enzyme activity unit is defined as the amount of enzyme in every 10,000 bacteria or cells that catalyzes the production of 1 nmol uric acid.

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

Calculation Parameters

Symbol	Meaning	Value or Unit
$V_{reaction\ total}$	Total volume of the reaction system	2.1×10^{-4}L
ε	Molar extinction coefficient of uric acid	1.22×10^4L/mol/cm
d	Optical path length of the 96-well UV plate	0.5 cm
10^9	Unit conversion factor	1 mol = 10^9nmol

Symbol	Meaning	Value or Unit
V_{sample}	Sample volume added	0.01 mL
$V_{sample\ total}$	Extraction Solution volume 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 formal measurement, select 2-3 samples with large expected differences for preliminary testing.
2. This 100T kit can measure 96 samples.
3. Required instruments and supplies are not included: microplate reader, benchtop centrifuge, adjustable pipettes, water bath or constant-temperature incubator, 96-well UV plate, homogenizer or mortar, cell ultrasonic disruptor, ice, and distilled water.

Visual Reference