Xanthine Oxidase (XOD) Assay Kit

Catalog No.: abx298945

Size: 96 tests

Storage: Store the Substrate Solution, Enzyme Reagent, Chromogenic Reagent A, and Chromogenic Reagent B in the dark at -20°C and the rest of the components -20°C.

Application: For detection of Xanthine Oxidase (XOD) activity in serum, plasma, tissue homogenates, and other biological fluids

Introduction

Xanthine Oxidase (XOD) is present in the liver, spleen and milk of mammals. XOD is an aerobic dehydrogenase which is an important enzyme involved in nucleic acid metabolism within the body. If hepatocytes are damaged, XOD will be released into the serum. Therefore, XOD is used an identifier for hepatocellular jaundice and obstructive jaundice. In hypoxia, xanthine dehydrogenase will form XOD which plays a vital role in the production of free radical. XOD catalyzes hypoxanthine to xanthine and superoxide anion free radicals.

Abbexa's Xanthine Oxidase (XOD) Assay Kit is a quick, convenient, and sensitive method for Xanthine Oxidase (XOD) activity. The product has an absorbance maxima at 550 nm. The intensity of the color is proportional to the Xanthine Oxidase (XOD) activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 20 ml
3. Substrate Solution: 1 ml
4. Enzyme Reagent: 1 ml
5. Chromogenic Reagent A: 1.6 ml
6. Chromogenic Reagent B: 1.6 ml
7. 1 mmol/L Standard Solution: 3.2 ml
8. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (550 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use.

- **Chromogenic Working Solution**: Mix 20 µl of Chromogenic Reagent A and 20 µl of Chromogenic Reagent B. The Chromogenic Working Solution should be prepared just before use. It can be stored in the dark for up to 1 hour.
- **Working Solution**: Prepare 180 µl of Working Solution for each well by mixing 147 µl of Buffer Solution, 6.5 µl of Substrate Solution, 6.5 µl of Enzyme Reagent and 20 µl of Chromogenic Working Solution. The Working Solution should be prepared just before use and stored in the dark.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 20 mg of tissue and wash with pre-chilled PBS (0.01 M, pH 7.4). For each 20 mg of tissue, add 180 µl of pre-chilled normal saline (0.9% NaCl). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Rat Plasma	1
Rat Serum	1
10% Mouse Kidney Tissue Homogenate	1
10% Rat Liver Tissue Homogenate	1
10% Rat Kidney Tissue Homogenate	1
10% Mouse Liver Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.
- The diluent is normal saline (0.9% NaCl).

B. Assay Procedure

1. Set standard, and sample tubes. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.
2. Standard curve preparation: Label 8 tubes with 1, 0.8, 0.7, 0.6, 0.4, 0.3, 0.2, 0 mmol/L. Dilute the 1 mmol/L Standard Solution with double distilled water to create the following concentrations: 0, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, and 1 mmol/L. See the table for serial dilution reference.

Concentration (mmol/L)	0	0.2	0.3	0.4	0.6	0.7	0.8	1
1 mmol/L Standard (µl)	0	40	60	80	120	140	160	200
Double distilled water	200	160	160	120	80	60	40	0

3. Add 20 µl the standard with the corresponding concentrations into the standard wells.
4. Add 20 µl of sample into the sample wells.
5. Add 180 µl of Working Solution into each well. Mix fully.
6. Measure the OD values of the sample wells at 550 nm with the microplate reader. These measurements should be recorded as A_1.
7. Incubate the plate at 37°C for 25 minutes.
8. Measure the OD values of the sample wells and standard wells at 550 nm with the microplate reader. These measurements should be recorded as A_2.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{550} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve (y = ax + b). Create the standard curve with graph software. The XOD concentration of the samples can be interpolated from the standard curve.

1. Serum and plasma samples:

One unit is defined as the amount of XOD in 1 L of serum or plasma samples that hydrolyzes the substrate to produce 1 µmol of H_20_2 in 1 minute at 37°C.

$$\text{XOD Activity (U/L)} = \frac{(\Delta A_{550} - b)}{a \times T} \times 1000 \times f$$

2. Tissues samples:

One unit is defined as the amount of XOD in 1 g of tissue that hydrolyzes the substrate to produce 1 µmol of H_20_2 in 1 minute at 37°C.

$$\text{XOD Activity (U/gprot)} = \frac{(\Delta A_{550} - b)}{a \times T} \times \frac{f}{C_{pr}} \times 1000$$

where:

ΔA_{550}	The change in OD of the sample ($\Delta A_{550} = A_2 - A_1$)
T	The time of reaction (25 minutes)
f	Dilution factor of the sample before testing
C_{pr}	The concentration of protein in sample (gprot/L)
1000	1 mmol/L = 1000 µmol/L
a	Gradient of the standard curve
b	Intercept of the standard curve