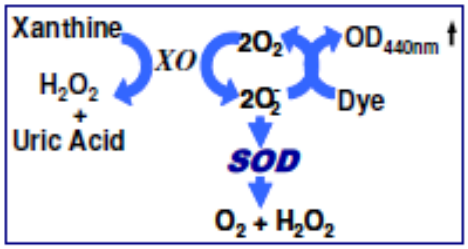
Superoxide Dismutase (SOD) Assay Kit (Colorimetric)

LS-K224-100 (100 Tests) • **Store at -20°C**

Introduction

Superoxide dismutases (SOD, EC1.15.1.1) are enzymes that catalyze the dismutation of superoxide into O_2 and H_2O_2. They are an important antioxidant defense in all cells exposed to O_2. There are three major families of superoxide dismutase: Cu/Zn, Fe/Mn, and the Ni type. Aberrant SOD activities have been linked to diseases such as amyotrophic lateral sclerosis, perinatal lethality, neural disorders and cancer.

This SOD assay provides a convenient colorimetric means for the quantitative determination of SOD enzyme activity in biological samples. In the assay, superoxide (O_2^-) is provided by xanthine oxidase (XO) catalyzed reaction. O_2^- reacts with a WST-1 dye to form a colored product. SOD scavenges the O_2^- thus less O_2^- is available for the chromogenic reaction. The color intensity (OD_{440nm}) is used to determine the SOD activity in a sample.



Key Features

- Sensitive and accurate. Linear detection range of 0.05 - 8 U/mL SOD.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Determination of SOD in blood, cell, tissue and other biological samples.

Components

Component	K224-100 100 Tests
Assay Buffer	20 mL
Diluent	20 mL
SOD Enzyme	120 µL
XO Enzyme	350 µL
Xanthine	600 µL
WST-1	600 µL

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Materials Not Supplied

Pipetting devices, tissue homogenizer, centrifuge and tubes, clear flat-bottom 96-well plates and plate reader.

Storage

The kit is shipped at room temperature. Store XO Enzyme at 2-8°C, and all other components at -20°C. Shelf life of 6 months after receipt.

Precautions

Reagents are for research use only. Normal precautions for laboratory reagent should be exercised while using the reagents.

Assay Procedure

Note: If not assayed immediately, samples can be stored at -80°C for one month. All samples can be diluted in 50 mM potassium phosphate, pH 7.4.

Tissue samples

Perfuse tissue with cold PBS to remove any red blood cells. Homogenize tissue at 5 mL/g in cold lysis buffer (50mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton X-100). Centrifuge at 12,000g for 5 minutes at 4°C. Use supernatant for total SOD assay.

Cell samples

Suspension cells: Centrifuge 1-2 x 10^6 cells at 800g for 2 minutes and discard supernatant. Wash cells with cold PBS, centrifuge, and discard the supernatant. Resuspend cells in 0.5 mL of cold lysis Buffer. After 10 min on ice, centrifuge at 12,000g for 5 min. Use supernatant for total SOD assay.

Adherent cells: Wash 1-2 x 10^6 cells cold PBS. Place dish on ice. Add 0.5 mL of cold lysis buffer. After 10 min on ice, collect cells/debris with a rubber policeman. Centrifuge the cell extract at 12,000g for 5 min. Use supernatant for total SOD assay.

Note: if it is desired to determine cytosolic and mitochondrial SOD activities separately, tissue/cell samples can be prepared according to Mattiazzi et al (2002). JBC 277: 29626-33.

Blood samples

Collect serum, or plasma (heparin, citrate or EDTA) using standard protocols. The erythrocyte pellet can be lysed in 5x volume of cold dH_2O; centrifuge at 12,000g for 5 min to pellet the erythrocyte membranes. Dilute serum/plasma 1:5, red cell lysate 1:100 prior to SOD assay.

Prior to assay, bring all reagents to room temperature (25°C). The Xanthine reagent may appear to be turbid. Briefly vortex this tube or gently heat the reagent for several minutes and vortex to mix thoroughly. Briefly centrifuge enzyme tubes, keep on ice during assay.

Standards

1. Mix 18µL SOD Enzyme with 320 µL Diluent to give 8U/mL SOD standard. Dilute standards as below.

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No	8U/ml SOD + Diluent	Standard (U/ml)
1	100 uL + 0 µL	8
2	80 µL + 20 µL	6.4
3	60 µL + 40 µL	4.8
4	40 µL + 60µL	3.2
5	18 µL + 82 µL	1.44
6	8 µL + 92 µL	0.64
7	4 µL + 96 µL	0.32
8	0 µL + 100 µL	0

Transfer 20 µL SOD standards to separate wells of a clear flat-bottom 96-well plate.

Transfer 20 µL samples to separate wells.

2. Prepare enough Working Reagent for the standard and sample wells. For each well, mix 160 µL Assay Buffer, 5 µL Xanthine and 5 µL WST-1. Transfer 160 µL Working Reagent to each well and tap plate to mix.

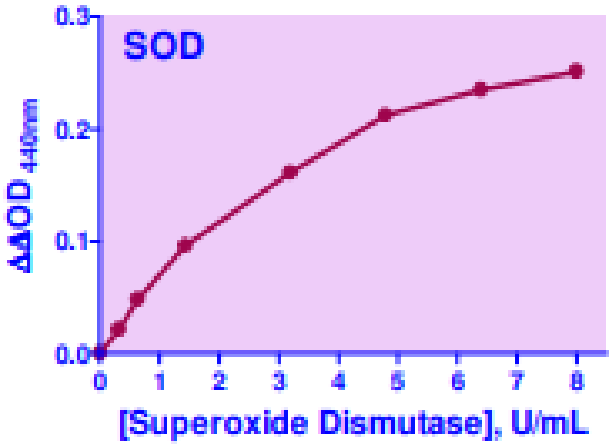
   For each well, dilute the XO Enzyme in Diluent: 3.5 µL XO enzyme with 18 µL Diluent. Quickly add 20 µL diluted XO enzyme to each assay well (use of a multi-channel pipettor is recommended). Tap plate to mix.

3. Immediately read OD_{440nm} (OD420-460nm) (OD_o). Incubate for 60 min at room temperature (25°C) in the dark. Read OD_{440nm} again (OD_{60}).

Calculations

1. For each standard and sample well, calculate $\Delta OD_{60} = OD_{60} - OD_o$.
2. Calculate $\Delta\Delta OD = \Delta OD_{std8} - \Delta OD$ for each standard and sample where ΔOD_{std8} is the ΔOD for Standard # 8 (the standard with no SOD activity and highest possible absorbance).
3. Plot the Standard Curve ΔΔOD vs [SOD](U/mL). Use the ΔΔOD for sample to determine SOD activity of sample from the standard curve.

Sample Data



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