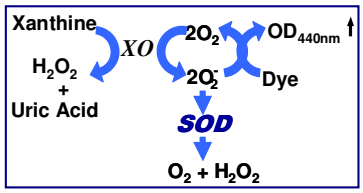
EnzyChrom™ Superoxide Dismutase Assay Kit (ESOD-100)

Quantitative Colorimetric Determination of SOD Activity

DESCRIPTION

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.

BioAssay Systems' 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 - 3 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.

KIT CONTENTS

Assay Buffer:	20 mL	**Diluent:**	20 mL
SOD Enzyme:	120 µL	**XO Enzyme:**	120 µL
Xanthine:	600 µL	**WST-1:**	600 µL

Storage conditions: The kit is shipped on ice. Store all reagents at -20°C. Shelf life of 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

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

1. 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 12,000*g* for 5 minutes at 4℃. Use supernatant for total SOD assay.

2. *Cell samples*.

Suspension cells: Centrifuge 1-2 x 10^6 cells at 800*g* 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 12,000*g* 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 12,000*g* 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.

3. 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 12,000*g* 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 vortext this tube before pipetting. Briefly centrifuge enzyme tubes, keep on ice during assay.

1. *Standards*. Mix 8µL SOD Enzyme with 392µL Diluent to give 3U/mL SOD standard. Dilute standards as below.

No	3U/mL SOD + Diluent	Standard (U/mL)
1	100 µL + 0 µL	3.0
2	80 µL + 20 µL	2.4
3	60 µL + 40 µL	1.8
4	40 µL + 60 µL	1.2
5	18 µL + 82 µL	0.54
6	8 µL + 92 µL	0.24
7	4 µL + 96 µL	0.12
8	0 µL + 100 µL	0.0

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 1:20 in Diluent. Quickly add 20 µL diluted XO enzyme to each assay well (use of a multi-channel pipettor is recommended). Tap plate to mix.

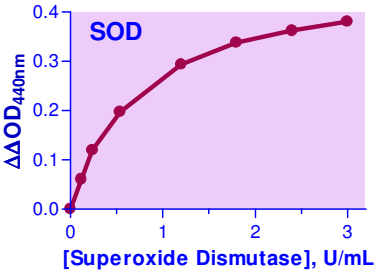
3. Immediately read OD_{440nm} (OD420-460nm) (OD_o). Incubate for 60 min at room temperature (25°C) in the dark. Read OD440nm again (OD_{60}).

CALCULATION

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 $\Delta\Delta OD$ *vs* [SOD](U/mL). Use the $\Delta\Delta OD$ for sample to determine SOD activity of sample from the standard curve.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



LITERATURE

1. Kuthan H, et al (1986). A spectrophotometric assay for superoxide dismutase activities in crude tissue fractions. Biochem J. 237: 175-80.

2. Ukeda H, et al (1997). Spectrophotometric assay for superoxide dismutase based on tetrazolium salt 3'--1--(phenylamino)-carbonyl--3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate reduction by xanthine-xanthine oxidase. Anal Biochem. 251: 206-9.

3. Janknegt PJ, et al (2007). A comparison of quantitative and qualitative superoxide dismutase assays for application to low temperature microalgae. J Photochem Photobiol B. 87: 218-26.