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Technical Bulletin

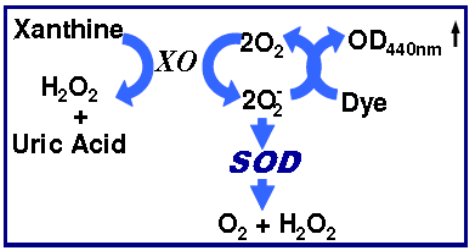
Superoxide Dismutase (SOD) Assay Kit

Catalogue number MAK528

Product Description

Superoxide dismutase (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.

The SOD Assay Kit 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_{440}) is used to determine the SOD activity in a sample.



The linear detection range of the kit is 0.05 - 3 U/mL SOD. The kit is suitable for SOD determination in blood, cell, tissue, and other biological samples.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 20 mL
  Catalogue Number MAK528A
- Diluent 20 mL
  Catalogue Number MAK528B
- SOD Enzyme 120 µL
  Catalogue Number MAK528C
- XO Enzyme 400 µL
  Catalogue Number MAK528D
- Xanthine 600 µL
  Catalogue Number MAK528E
- WST-1 600 µL
  Catalogue Number MAK528F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories
  (For example., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- Tissue Homogenizer
- 1.5 mL Centrifuge tubes
- 50 mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton™ X-100. (Cold Lysis Buffer)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature (25 °C) prior to use.

Xanthine Reagent: The reagent may appear to be turbid. Briefly vortex this tube before pipetting.

SOD and XO Enzyme: Briefly centrifuge enzyme tubes, keep on ice during assay.

Procedure

All Samples and Standards should be run in duplicate.

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.

Note: Prior to assay, bring all reagents to room temperature (25 °C). The Xanthine reagent may appear to be turbid. Briefly vortex this tube before pipetting. Briefly centrifuge enzyme tubes, keep on ice during assay.

Sample Preparation

Tissue:

1. Perfuse tissue with cold PBS to remove any red blood cells.
2. Use 5 mL/g of cold lysis buffer (50 mM potassium phosphate, 0.1 mM EDTA, 0.5% Triton™ X-100) to homogenize the tissue.
3. Centrifuge at 12,000 × g for 5 minutes at 4 °C.
4. Remove supernatant for assay.

Cell Samples:

For Suspension cells:

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

For Adherent cells:

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

For Blood Samples:

1. Collect serum, or plasma (heparin, citrate or EDTA) using Standard protocols.
2. The erythrocyte pellet can be lysed in 5x volume of cold dH_2O.
3. Centrifuge at 12,000 × *g* for 5 minutes to pellet the erythrocyte membranes.
4. Dilute serum/plasma 1:5, red cell lysate 1:100 prior to SOD assay.

Transfer 20 µL samples to separate wells.

Standard Curve Preparation

Prepare 3 U/mL SOD Standard by mixing 8 µL SOD Enzyme with 392 µL Diluent. Dilute the Standards as described in Table 1.

Table 1.
Preparation of Standards

Well	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 90 µL Working Reagent into each reaction (and control) well. Tap plate to mix.

Working Reagents

Prepare enough working reagents for the number of assays to be performed. For each well, prepare 170 µL of Working Reagent and Blank Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagent.

Reagent	Working Reagent
Assay Buffer	160 µL
Xanthine	5 µL
WST-1	5 µL

Transfer 160 µL Working Reagent to each well and tap plate to mix.

Assay Reaction

1. Prepare enough XO enzyme by mixing 4 µL XO Enzyme in 20 µL Diluent per well.
2. Quickly add 20 µL diluted XO enzyme to each assay well using a multi-channel pipettor.
3. Tap plate to mix.

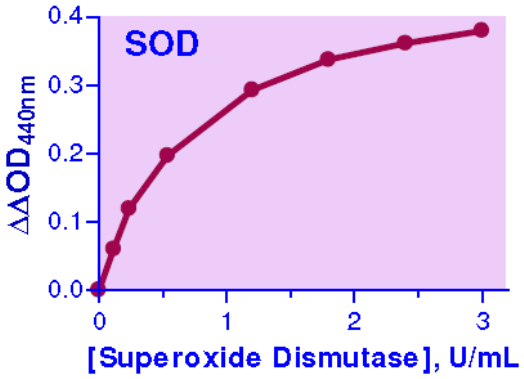
Measurement

1. Immediately read OD_{440nm} ($OD_{420-460nm}$) (OD_0).
2. Incubate for 60 min at room temperature (25 °C) protected from light.
3. Read OD_{440nm} again (OD_{60}).

Results

1. For each Standard and Sample well, calculate ΔOD_{60} by subtracting OD value at zero min from OD value read at 60 min (OD_0 and OD_{60}, respectively)
   $\Delta OD_{60} = OD_{60} - OD_0$
2. Calculate ΔΔOD by subtracting ΔOD from ΔOD_{BLANK} for each Standard and Sample where ΔOD_{BLANK} is the ΔOD for Standard #8 (no SOD activity and highest possible absorbance.)
   $\Delta\Delta OD = \Delta OD_{BLANK} - \Delta OD$
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.

Figure 1:
Typical Standard curve for Superoxide Dismutase



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