

Technical Bulletin

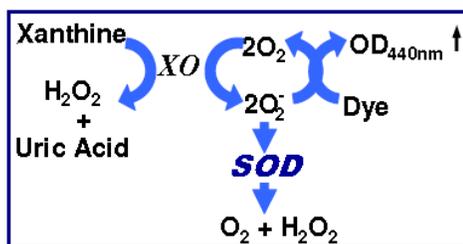
Superoxide Dismutase (SOD) Assay Kit

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 μ L
• Catalogue Number MAK528C	
• XO Enzyme	400 μ L
• Catalogue Number MAK528D	
• Xanthine	600 μ L
• Catalogue Number MAK528E	
• WST-1	600 μ L
• 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 $-20\text{ }^{\circ}\text{C}$.

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 × 10⁶ 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 × 10⁶ 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 dH₂O.
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 20 µL of each SOD standard to separate wells.

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 $\text{OD}_{440\text{nm}}$ ($\text{OD}_{420-460\text{nm}}$) (OD_0).
2. Incubate for 60 min at room temperature (25 $^{\circ}\text{C}$) protected from light.
3. Read $\text{OD}_{440\text{nm}}$ 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)

$$\Delta\text{OD}_{60} = \text{OD}_{60} - \text{OD}_0$$

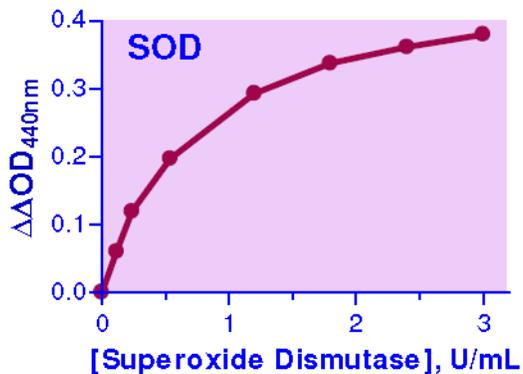
2. Calculate $\Delta\Delta\text{OD}$ by subtracting ΔOD from $\Delta\text{OD}_{\text{BLANK}}$ for each Standard and Sample where $\Delta\text{OD}_{\text{BLANK}}$ is the ΔOD for Standard #8 (no SOD activity and highest possible absorbance.)

$$\Delta\Delta\text{OD} = \Delta\text{OD}_{\text{BLANK}} - \Delta\text{OD}$$

3. Plot the Standard Curve $\Delta\Delta\text{OD}$ vs $[\text{SOD}](\text{U}/\text{mL})$. Use the $\Delta\Delta\text{OD}$ for Sample to determine SOD activity of Sample from the Standard curve.

Figure 1:

Typical Standard curve for Superoxide Dismutase



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