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DATA SHEET

Version: 04
Revision date: 03/02/2026

1. Identification

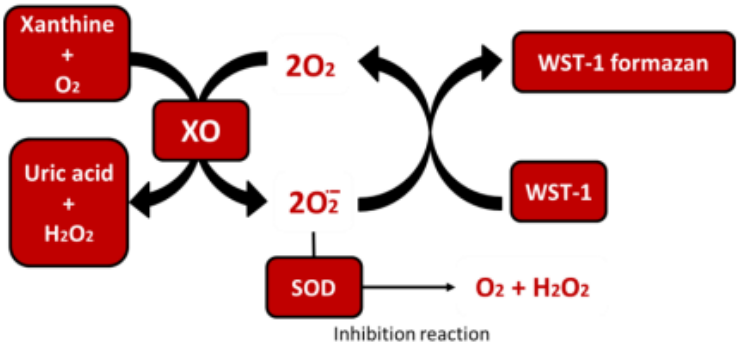
Product name **SOD Assay Kit**

100 assays

Cat. No: **CA061**

2. Description

Superoxide dismutase (SOD) catalyzes the breakdown of superoxide radicals and provides the first line of defense against oxygen toxicity. In order to determine the SOD activity, several direct and indirect methods have been developed. In the **SOD Assay Kit**, superoxide ions are generated from the conversion of xanthine and O2 to uric acid and H2O2 by Xanthine Oxidase (XO). The superoxide anion then coverts the tetrazolium salt WST-1 to the coloured product WST-1 formazan. Absorbance is then measured at 450 nm using a standard microplate reader. Addition of SOD to this reaction reduces superoxide ion levels, thereby lowering the rate of WST-1 formazan formation. SOD activity in the experimental sample is measured as the percent inhibition of the rate of WST-1 formazan formation.



3. Composition & Storage

Item	Quantity	Storage
WST-1 Reagent!	1 mL	4°C
SOD assay buffer	20 mL	4°C
SOD dilution buffer	10 mL	4°C
SOD enzyme solution	30 µL	4°C
SOD standard (500 U/mL)	50 µL	-20°C

! WST-1 reagent is light sensitive and should be maintained in amber tubes.

4. Aplications

Quantitative determination of Superoxide Dismutase (SOD) enzyme activity.

5. Features

- Easy to use.
- Just a few minutes procedure (~30 minutes).
- Suitable for use with Serum, Plasma, Tissue Extracts, Cell Lysate, Cell culture media and Other biological fluids

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6. Working Solution Preparation

1. **WST working solution**: Dilute 1 ml of WST Solution with 19 ml of SOD assays Buffer.
2. **Enzyme working solution**: Centrifuge the Enzyme Solution tube for 5 sec. Mix by pipetting, and dilute 25 µl of Enzyme Solution with 2.5 ml of Dilution Buffer.

7. Sample Preparation

Choose the appropriate protocol to Process Sample before proceeding to SOD assay. Please note that samples should be kept on ice to maintain enzyme activity.

Sample	Protocol
Tissue Homogenate/ Cell Lysate	1. Homogenize tissue or lyse cells in ice-cold 0.1 M Tris/HCl, pH 7.4 containing 0.5 % Triton X-100, 5 mM β-ME, 0.1 mg/ml PMSF. 2. Centrifuge the crude tissue homogenate/cell lysate at 14000 x g for 5 minutes at 4˚C and discard the cell debris. 3. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80˚C. *The supernatant contains total SOD activity from cytosolic and mitochondria.* **Recommended amount of starting material:** Cells:1-5 x 106 Tissue: ≈10 mg
Plasma	1. Collect blood using an anticoagulant such as heparin, citrate, or EDTA. 2. Centrifuge the blood at 700-1,000 x g for 10 min at 4˚C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80˚C. The plasma sample will be stable for at least one month.
Serum	1. Collect blood without anticoagulant. 2. Allow blood to clot for 30 min at 25˚C. 3. Centrifuge the blood at 2,000 x g for 15 min at 4˚C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice until assaying or freeze at -80˚C. The sample will be stable for at least one month.

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8. S O D Assay

*Refer to Table I of the amount of solution in each well. If you are using a SOD standard, set up wells for it in the same manner as the sample. Keep samples on ice to maintain enzyme activity.

1. Add **20 µL** of dilution prepared for the **Standard Curve**.*
2. Add **20 µl of Sample Solution** to each sample well and blank 2 well and add 20 µl H_2O to each Blank 1
3. and Blank 3 well (See Table I).
4. Add **200 µl of the WST Working Solution** to each well.
5. Add **20 µl of Dilution Buffer** to each Blank 2 and Blank 3 well.
6. Add **20 µl of Enzyme Working solution** to each sample and Blank 1 well, mix thoroughly.
7. Incubate plates at 37°C for 20 minutes.
8. Read the absorbance at 450 nm

Table I : Amount of each solution for sample, blank 1, 2 and 3

	Sample	Blank 1	Blank2*	Blank 3
Sample solution	20 µl		20 µl	
ddH2O		20 µl		20 µl
WST working solution	200 µl	200 µl	200 µl	200 µl
Enzyme working solution	20 µl	20 µl		
Dilution buffer			20 µl	20 µl

****Standard curve:** In microcentrifuge tubes, prepare the standard curve by serial dilutions of the SOD Standard in SOD Dilution Buffer over the validated linear range of 0.1–10 U/mL.*

9. Calculation

Calculate the SOD activity (inhibition rate %) using the following equation:

$$\text{SOD Activity (Inhibition rate \%)} = \frac{(A_{blank1}-A_{blank3})-(A_{sample}-A_{blank2})}{(A_{blank1}-A_{blank3})} \times 100$$

9. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

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