Total Superoxide Dismutase Assay Kit (WST-8 Method)

Introduction

Superoxide Dismutase (SOD) is a critical antioxidant defense enzyme that catalyzes the dismutation of the superoxide anion radical ($O_2^{\cdot -}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). By scavenging excess superoxide radicals, SOD protects cells against oxidative damage. Aberrant SOD activity is closely associated with aging, inflammation, neurodegenerative diseases, and various chronic conditions. Thus, accurate measurement of SOD activity is essential for evaluating oxidative stress status, studying antioxidant mechanisms, and disease diagnosis.

This kit employs the WST−8 method, where xanthine oxidase (XO) generates superoxide anions to reduce WST−8 into a soluble formazan dye. SOD inhibits this reaction dose−dependently. Therefore, SOD activity is inversely proportional to formazan production. Compared to traditional NBT or Cytochrome C methods, this kit offers higher sensitivity, a broader linear range, superior formazan solubility, and excellent stability, making it ideal for high−throughput screening. Furthermore, the assay buffer is supplemented with catalase to eliminate interference from endogenous H_2O_2, ensuring reliable results.

Components and Storage

Components \ Size	100 Assays	250 Assays	Storage
SOD Assay Buffer	100 mL	250 mL	-20°C
WST-8	800 µL	2 mL	-20°C away from light
Enzyme Solution	100 µL	250 µL	-20°C
Assay Starting Buffer (40X)	60 µL	150 µL	-20°C
SOD Standard	30 µL	75 µL	-20°C
Shipping: Blue ice	**Shelf life: 6 months**		

Protocol

1. Sample preparation

   1) Cell samples:

      a) For adherent cells, discard the culture medium and wash once with pre-chilled PBS or saline. Homogenize cells in 100-200 µL of SOD Assay Buffer per 1 × 10^6 cells. Centrifuge at 12,000 × *g* for

10 min at 4°C. Collect supernatant and keep it on ice.

b) For suspension cells, collect cells by centrifugation at 600 × *g* for 5 min. Wash once with cold PBS. Homogenize in 100-200 µL of SOD Assay Buffer per 1 × 10^6 cells. Centrifuge at 12,000 × *g* for 10 min at 4°C. Collect supernatant and keep it on ice.

2) Tissue samples: Perfuse the animal with saline containing 0.16 mg/mL heparin sodium to remove blood. Harvest tissue. Homogenize tissue in 100 µL of SOD Assay Buffer per 10 mg tissue on ice. Centrifuge at 12,000 × *g* for 10 min at 4°C. Collect supernatant and keep it on ice.

3) Plasma or red blood cells: Collect blood in an anticoagulant tube and invert to mix. Centrifuge at 600 × *g* for 10 min at 4°C. Transfer the supernatant to a new tube and dilute with saline or SOD Assay Buffer to prepare the plasma sample. For red blood cells, refer to the suspension cell preparation method.

Note:

a) After sample preparation, quantify protein using the BCA Protein Assay Kit (Cat# K4101). Typically, 10-20 µg of protein from cells or tissues corresponds to 1 unit of SOD activity; this varies by sample type and serves as a preliminary reference. Preparing 20-100 µL of protein sample is generally sufficient for detection.

b) Prepared samples should ideally be tested on the same day. If immediate testing is not feasible, store samples at -80°C and use within 1 month. Avoid repeated freeze-thaw cycles, as they may reduce SOD activity.

2. Sample dilution: Samples can be diluted with SOD Assay Buffer referring to the table below.

Sample	Dilution factor
10% of mouse liver	10-80
10% of mouse kidney	10-80
Mouse plasma	2-4
Cell homogenate	0-4

3. Kit preparation

1) Preparation of reaction working solution: Prepare the reaction working solution according to the table below. Store on ice or at 4°C and use the same day. Fresh preparation prior to use is optimal. Ensure WST−8 is fully thawed prior to use. Briefly centrifuge the Enzyme Solution, then mix well.

Number of Samples to Test	1	10	20
SOD Assay Buffer	151 µL	1510 µL	3020 µL
WST-8	8 µL	80 µL	160 µL
Enzyme Solution	1 µL	10 µL	20 µL
Total volume	160 µL	1600 µL	3200 µL

2) Preparation of Assay Starting Buffer (1X): Thaw the Assay Starting Buffer (40X) in advance. Subsequently, dilute the appropriate amount of Assay Starting Buffer (40X) with SOD Assay Buffer to obtain Assay Starting Buffer (1X). Store Assay Starting Buffer (1X) on ice or at 4°C and use the same day. Fresh preparation prior to use is optimal.

3) (Optional) Preparation of SOD Standard: Dilute an appropriate amount of SOD Standard with SOD Assay Buffer into a series of concentrations. Upon first use, it is recommended to prepare concentrations of: 100

U/mL, 50 U/mL, 20 U/mL, 10 U/mL, 5 U/mL, 2 U/mL, 1 U/mL.

Note: Different concentrations of SOD standard should be prepared fresh and used immediately; otherwise, enzyme activity may decline. The standard is not mandatory for detecting SOD activity but can serve as a positive control or reference for SOD activity quantification.

4. SOD activity detection

1) Group the samples referring to the table below and add samples and other solutions sequentially. After adding Assay Starting Buffer (1X), mix thoroughly. Once Assay Starting Buffer (1X) is added, the reaction starts. Operate on ice or use a multi-channel pipette to minimize errors caused by different addition times across wells.

	Samples/Standards	**Blank Control 1**	**Blank Control 2**	**Blank Control 3 (optional)**
Samples/Standards	20 μL	-	-	20 μL
SOD Assay Buffer	-	20 μL	40 μL	20 μL
Reaction working solution	160 μL	160 μL	160 μL	160 μL
Assay Starting Buffer (1X)	20 μL	20 μL	-	-

Note: If the sample has color or contains antioxidant substances, set up Blank Control 3; otherwise, it is not needed.

2) Incubate at 37°C for 30 min.

Note: There is no significant difference in detected SOD activity when incubating for 25-35 min, but to ensure consistency, a 30-min incubation is recommended.

3) Measure the absorbance at 450 nm. If a 450 nm filter is unavailable, a 420-480 nm filter can also be used. Simultaneously, 600 nm can be set as a reference wavelength, using the reading at 450 nm minus the reading at the reference wavelength as the actual reading.

5. SOD activity calculation

1) Calculate the Inhibition Rate:

Inhibition Rate = $[(A_{blank1} - A_{blank2}) - (A_{sample} - A_{blank3})] / (A_{blank1} - A_{blank2}) \times 100\%$

If blank control 3 is not needed, the formula can be simplified to

Inhibition Rate = $(A_{blank1} - A_{sample}) / (A_{blank1} - A_{blank2}) \times 100\%$

If the calculated inhibition rate is < 30% or > 70%, it is recommended to retest, trying to keep the sample's inhibition rate within the 30-70% range. If the sample inhibition rate is too high, dilute the sample appropriately; if the inhibition rate is too low, prepare a higher concentration sample.

2) Definition of SOD activity unit: Under the xanthine oxidase-coupled reaction, when the inhibition rate is 50%, the SOD activity in the reaction system is defined as one unit. Calculate SOD activity according to this definition:

Sample SOD Activity = Detected System SOD Activity = Inhibition Rate / (1 - Inhibition Rate) units

For example, when the inhibition rate is 50%, SOD activity in the sample = 50% units / (1-50%) units = 1 unit

3) For tissue or cell homogenate, the SOD activity units can be converted to U/g or U/mg protein based on the sample's protein concentration and dilution factor. If the sample is a red blood cell extract, it can be converted to U/g or U/mg hemoglobin based on the hemoglobin content.

4) Reference method for calculating SOD enzyme activity: Generate a standard curve of inhibition rate using the SOD Standard. Then, calculate the SOD activity of samples based on their inhibition rates using the standard curve. This method requires that the standard's activity data is reliable and does not decrease due to storage issues. This method is for reference only.

5) Kinetic method: SOD activity can also be calculated using the kinetic method. That is, during the 37°C incubation, absorbance can be measured continuously for 30 min. Calculate the inhibition rate based on the slope of absorbance change over 30 min. The kinetic method provides more precise results but is relatively complex; generally, the endpoint method is sufficient.

$$\text{Inhibition Rate} = [(\text{Slope}_{\text{blank1}} - \text{Slope}_{\text{blank2}}) - (\text{Slope}_{\text{sample}} - \text{Slope}_{\text{blank3}})] / (\text{Slope}_{\text{blank1}} - \text{Slope}_{\text{blank2}}) \times 100\%$$

Note

1. If the kit cannot be used up within three runs, it is recommended to aliquot the WST-8 upon first use to avoid degradation caused by repeated freeze-thaw cycles.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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