# Technical Manual

# Total Superoxide Dismutase (T-SOD) Activity Assay Kit (WST-1 Method)

- Catalogue Code: MAES0034
- Size: 96T
- Research Use Only

# 1. Key features and Sample Types

## Detection method:

Colorimetric method

## Specification:

96T

## Range:

0.2 -14.4 U/mL

## Sensitivity:

0.2 U/mL

## Storage:

2-8°C and -20°C for 12 months

## Expiry:

See Kit Label

## Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 12 months.

Do not use components from different batches of kit.

## 2. Background

According to the literature, superoxide dismutase exists in all oxygen-metabolizing cells to protect cells from excessive superoxide. Under the action of SOD, two superoxide anions were converted to oxygen and hydrogen peroxide. The reaction principle is as follows:In mammals, there are three different forms of SOD: CuZn-SOD, Mn-SOD and EC-SOD (an extracellular form of SOD). Cu-Zn SOD exists in the cytoplasmic and mitochondrial membrane spaces of the cells, while Mn-SOD is located in the mitochondrial matrix.

## 3. Intended Use

This kit can be used to measure total superoxide dismutase (T-SOD) activity in serum, plasma, pleural effusion, ascites, urine, cells, various animal and plant tissues samples.

## 4. Detection Principle

The activity of SOD was measured by WST-1 method in this kit and the principles of the WST-1 is as follows. Xanthine Oxidase (XO) can catalyze WST-1 react with O2•- to generate a water-soluble formazan dye. SOD can catalyze the disproportionation of superoxide anions, so the reaction can be inhibited by SOD, and the activity of SOD is negatively correlated with the amount of formazan dye. Therefore, the activity of SOD can be determined by the colorimetric analysis of WST-1 products.

## 5. Kit components & storage

| Item | Specification | Storage |
|---|---|---|
| **Buffer Solution** | 24 mL × 1 vial | 2-8°C, 12 months |
| **Substrate Solution** | 0.14 mL × 1 vial | 2-8°C, 12 months, avoid direct sunlight |
| **Enzyme Stock Solution** | 0.3 mL ×1 vial | -20°C, 12 months |
| **Enzyme Diluent** | 1.5 mL × 2 vials | 2-8°C, 12 months |
| **Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (520-540 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

## 6. Assay Notes:

1. The Inhibition ratio of SOD should be 25%-65%.

2. Prevent the formulation of bubbles when adding the liquid to the microplate.

3. Superoxide is formed immediately after substrate application solution is added. The multichannel pipeter is recommended to shorten the time and reduce the error between wells.

## 7. Reagent preparation:

1. Enzyme Stock Solution should melt slowly on ice. It is recommended to aliquot the enzyme stock solution into smaller quantities for optimal storage. Prevent repeated freeze-thaw cycles. Bring other reagents to room temperature before use.

2. Preparation of **substrate application solution:** Mix the buffer solution and substrate solution at the ratio of 200:1 thoroughly. Prepare the fresh solution before use and the unused substrate application solution can be stored at 2~8°C for 7 days.

3. Preparation of **enzyme working solution:** Mix the enzyme stock solution and enzyme diluent at the ratio of 1:10 thoroughly. (Note: Please operate on the ice box.) Prepare the fresh solution before use and the unused enzyme working solution can be stored at 2-8°C for 3 days.

## 8. Sample Preparation

**Sample requirements:** The samples should not contain SDS, Tween20, NP-40, Triton X-100 and other detergents, and should not contain DTT, 2-merhydryl ethanol and other reducing reagents.

**1. Serum sample:**
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

**2. Plasma sample:**
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

**3. Urine:**
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

**4. Cell sample:**

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number ($2\times10^6$): PBS (0.01 M, pH 7.4) (μL) =1: 300. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

**5. Tissue sample:**

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

## Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

## Dilution of Samples:

The optimal sampling volume are different for different species, the SOD also are different for different samples. It is recommended to take 2~3 samples to do a pre-experiment, diluting a series of diluent and determine the dilution factor when the SOD inhibition ratio is 25%~65% before formal experiment.

The recommended dilution factor for different samples is as follows (for reference only).

| Sample Type: | Dilution Factor |
|---|---|
| Human serum | 3-5 |
| Rat serum | 20-30 |
| Urine | 1 |
| Human hydrothorax | 2 |
| Cell culture supernatant | 2-3 |
| 10% Rat liver tissue homogenization | 340-370 |
| 10% Rat heart tissue homogenization | 80-100 |
| 10% Rat kidney tissue homogenization | 100-120 |
| 10% Rat brain tissue homogenization | 50-100 |
| HepG2 cells homogenization (3 mgprot/mL) | 30-40 |
| 10% Plant tissue homogenization | 5-10 |
| Rat serum | 20-30 |

**Note:** The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

# 9. Assay Protocol

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** 450 nm

## Plate Set Up:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | A | A | S13 | S14 | S29 | S30 | S45 | S46 | S61 | S62 | S77 | S78 |
| **B** | B | B | S15 | S16 | S31 | S32 | S47 | S48 | S63 | S64 | S79 | S80 |
| **C** | S1 | S2 | S17 | S18 | S33 | S34 | S49 | S50 | S65 | S66 | S81 | S82 |
| **D** | S3 | S4 | S19 | S20 | S35 | S36 | S51 | S52 | S67 | S68 | S83 | S84 |
| **E** | S5 | S6 | S21 | S22 | S37 | S38 | S53 | S54 | S69 | S70 | S85 | S86 |
| **F** | S7 | S8 | S23 | S24 | S39 | S40 | S55 | S56 | S71 | S72 | S87 | S88 |
| **G** | S9 | S10 | S25 | S26 | S41 | S42 | S57 | S58 | S73 | S74 | S89 | S90 |
| **H** | S11 | S12 | S27 | S28 | S43 | S44 | S59 | S60 | S75 | S76 | S91 | S92 |

**Note:** A, Control well; B, $Blank_{Control}$ well; S1-S92, sample wells.

# 10. Operation Steps

1. **Control well:** Add 20 µL of double distilled water and 20 µL of enzyme working solution.
   **$Blank_{Control}$ well:** Add 20 µL of double distilled water and 20 µL of enzyme diluent.
   **Sample well:** Add 20 µL of sample and 20 µL of enzyme working solution.

2. Add 200 µL of substrate application solution with a multi-channel pipettor into each well and mix fully.

3. Incubate at 37°C for 20 min. Measure the OD values of each well at 450 nm with microplate reader.

## Operation Table

| | Control well | $Blank_{Control}$ well | Sample well |
|---|---|---|---|
| **Sample (µL)** | | | 20 |
| **Double distilled water (µL)** | 20 | 20 | |
| **Enzyme working solution (µL)** | 20 | | 20 |
| **Enzyme diluent (µL)** | | 20 | |
| **Substrate application solution (µL)** | 200 | 200 | 200 |
| Mix fully for 5 s with microplate reader. Incubate at 37°C for 20 min. Measure the OD values of each well at 450 nm with microplate reader. | | | |

# 11. Calculations

**Definition:** When SOD inhibition ratio in this reaction system reach 50%, the corresponding enzyme level is 1 SOD activity unit (U).

$$i = \frac{(A_{Control}-A_{Blank_{Control}})-(A_{Sample}-A_{Blank_{Control}})}{A_{Control}-A_{Blank_{Control}}} \times 100\%$$

**1. Serum (plasma), culture supernatant and other liquid sample:**

$$\text{SOD activity (U/mL)} = i \div 50\% \times \frac{V_1}{V_2} \times f$$

**2. Tissue and cells sample:**

$$\text{SOD activity (U/mgprot)} = i \div 50\% \times \frac{V_1}{V_2} \times f \div C_{pr}$$

**i:** Inhibition ratio of SOD (%)
**$V_1$:** The total volume of reaction, 240 μL
**$V_2$:** The volume of sample added to the reaction, 20 μL
**f:** Dilution factor of sample before tested
**$C_{pr}$:** Protein concentration of sample, mgprot/mL

# 12. Performance Characteristics

| | |
|---|---|
| **Detection Range** | 0.2 -14.4 U/mL |
| **Sensitivity** | 0.2 U/mL |
| **Average recovery rate (%)** | 97 |
| **Average inter-assay CV (%)** | 3.7 |
| **Average intra-assay CV (%)** | 2.9 |

## Analysis

Take human serum, dilute for 4 times with PBS, then take 0.02 mL of diluted sample, carry the assay according to the operation table.

**The results are as follows:**

The average OD value of the control is 0.608, the average OD value of $Blank_{Control}$ is 0.048, the average OD value of sample is 0.388, and the calculation result is:

$$\textbf{Inhibition ratio of SOD (\%)} = \frac{(0.608-0.048)-(0.388-0.048)}{(0.608-0.048)} \times 100\% = 39.29\%$$

$$\textbf{SOD activity (U/mL)} = 39.29\% \div 50\% \times \frac{0.24}{0.02} \times 5 = 47.15 \text{ (U/mL)}$$



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## Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.



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