Technical Manual

Total Superoxide Dismutase (T-SOD) Activity Assay Kit (Hydroxylamine Method)

- Catalogue Code: MAES0033
- Size: 100 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

4.7-166 U/mL

Sensitivity:

4.7 U/mL

Storage:

2-8°C and -20°C for 6 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 6 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, urine, cells, cell culture supernatant and tissue samples.

4. Detection Principle

The superoxide anion free radical (O2•-) can be produced by xanthine and xanthine oxidase reaction system, O2•- oxidize hydroxylamine to form nitrite, it turn to purple under the reaction of developer. When the measured samples containing SOD, the SOD can specifically inhibit superoxide anion free radical (O2•-). The inhibitory effect of SOD can reduce the formation of nitrite, the absorbance value of sample tube is lower than control tube. Calculate the SOD of sample according to the computational formula.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	12 mL × 1 vial	2-8°C, 6 months
Nitrosogenic Agent	12 mL × 1 vial	2-8°C, 6 months
Substrate Solution	12 mL × 1 vial	2-8°C, 6 months
Enzyme Stock Solution	0.6 mL × 1 vial	-20°C, 6 months
Enzyme Diluent	12 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent A	Lyophilized, 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent B	Lyophilized, 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent C	60 mL × 1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (550 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:

The time of incubation is 40 min, the time of incubation can be extended to 45 min when the room temperature is lower than 20°C. The temperature (37°C) of incubation should be stable.

7. Reagent Preparation:

1. Preparation of **buffer working solution:** Dilute the buffer solution with double distilled water at a ratio of 1:9 before use. Prepared solution can be stored at 2-8°C for 3 months.

2. Preparation of **enzyme stock application solution:** Dilute enzyme stock solution with enzyme diluent at a ratio of 1:19. Prepare the fresh solution before use. Unused solution can be stored at 2-8°C for 3 days.

3. Preparation of **chromogenic agent A application solution:** Dissolve a vial of chromogenic agent A powder with 70-80°C double distilled water to a final volume of 90 mL. It can be store at 2-8°C and avoid direct sunlight for 3 months.

4. Preparation of **chromogenic agent B application solution:** Dissolve a vial of chromogenic agent B powder with double distilled water to a final volume of 90 mL. It can be store at 2-8°C and avoid direct sunlight for 1 months.

5. Preparation of **chromogenic agent:** Prepare chromogenic agent at ratio of chromogenic agent A application solution: chromogenic agent B application solution: chromogenic agent C =3:3:2. Prepare the fresh solution before use and the prepared chromogenic agent can be stored at 2-8°C in the dark.

8. Sample Preparation

Sample requirements: Samples should not contain decontamination agents such as SDS, Tween-20, NP-40, Triton X-100, nor reductive reagents such as DTT, 2-mercaptoethanol.

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at-80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant), centrifuge at 1000-2000 g 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) and preserve on ice before 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 and preserve on ice before 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 (2×10^6): PBS (0.01 M, pH 7.4) (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before 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. Use filter paper to absorb excess water 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 and preserve on ice before 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 Inhibition ratio of this kit is 15-55%, the optimal inhibition ratio is 25-45%. When the inhibition ratio is 30-40%, the corresponding sampling volume is the optimal sampling volume.

$$\text{Inhibition ratio} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100\%$$

If inhibition ratio is more than 55%, dilute the sample and then carry the assay. If inhibition ratio is less than 15%, increase the dilution multiple.

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

Sample Type:	Dilution Factor:	The volume of sample
HepG2 supernatant	1	50 µL
HepG2 cell	8-10	25 µL
Mouse serum	3-5	20 µL
10% Mouse liver tissue homogenate	40-60	20 µL
10% Rat kidney tissue homogenate	15-20	20 µL
Human urine	1	25 µL

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: 550 nm

10. Operation Steps

1. **Sample tube:** add 1 mL of buffer working solution and a* mL sample to the sample tubes.
   Control tube: add 1 mL of buffer working solution and a* mL double distilled water to the control tubes.

2. Add 0.1 mL of nitrosogenic agent, 0.1 mL of substrate solution, 0.1 mL of enzyme stock application solution successively into the tubes of step 1.

3. Mix fully with a vortex mixer, incubate for 40 min at 37°C.

4. Add 2 mL of chromogenic agent into the tubes of Step 3.

5. Mix fully and stand for 10 min at room temperature.

6. Set to zero with double distilled water and measure the OD value of each tube at 550 nm with 1 cm optical path quartz cuvette.

7. **Note:** If the optimal sampling volume (a*) is the same, only one control tube need to be assay.

Operation Table

	Sample tube	Control tube
Buffer working solution (mL)	1.0	1.0
Sample (mL)	a*	
Double distilled water(mL)		a*
Nitrosogenic agent (mL)	0.1	0.1
Substrate solution (mL)	0.1	0.1
Enzyme stock application solution (mL)	0.1	0.1
Mix fully with a vortex mixer, incubate for 40 min at 37°C.		
Chromogenic agent	2.0	2.0
Mix fully and stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD value of each tube at 550 nm with 1 cm optical path quartz cuvette.		

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11. Calculations

1. Serum (plasma) and other liquid sample:

Definition: The amount of SOD when the inhibition ratio reaches 50% in 1 mL reaction solution is defined as 1 SOD activity unit (U).

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

2. Tissue and cell sample:

Definition: The amount of SOD when the inhibition ratio reaches 50% of 1 mg tissue protein in 1 mL reaction solution is defined as 1 SOD activity unit (U).

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

i: Inhibition ratio of SOD (%), $i = \frac{A_1-A_2}{A_1} \times 100\%$

A_1: the OD value of control tube at 550 nm

A_2: the OD value of sample tube at 550 nm

V_1: the total volume of reaction solution, mL

V_2: the volume of sample added into the reaction system, mL

f: dilution factor of sample before tested

C_{pr}: concentration of protein in sample, mgprot/mL

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12. Performance Characteristics

Detection Range	4.7-166 U/mL
Sensitivity	4.7 U/mL
Average recovery rate (%)	105
Average inter-assay CV (%)	6.3
Average intra-assay CV (%)	2.8

Analysis

Take 10% rat liver tissue homogenate and dilute for 10 times with PBS (0.01 M, pH 7.4), take 10 μL diluted sample, carry the assay according to the operation table.

The results are as follows:

The average OD value of the control tube is 0.343, the average OD value of the sample tube is 0.212, the concentration of protein in sample is 11.61 mgprot/mL, and the calculation result is:

$$\textbf{T-SOD activity (U/}\textit{\textbf{mgprot}}\textbf{)} = \left(\frac{0.343-0.212}{0.343}\right) \div 50\% \times \frac{3.31}{0.01} \times 10 \div 11.61$$

$$= 217.77 \text{ U/mgprot}$$

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.

Notes:

Notes:

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