AssayGenie

Technical Manual

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

- Catalogue Code: MAES0032
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

2.4-61 U/mL

Sensitivity:

2.4 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	1.2 mL × 1 vial	2-8°C, 6 months
Nitrosogenic Agent	1.2 mL × 1 vial	2-8°C, 6 months
Substrate Solution	1.2 mL × 1 vial	2-8°C, 6 months
Enzyme Stock Solution	30 µL × 1 vial	-20°C, 6 months
Enzyme Diluent	1.2 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	6 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (540-560 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 prepared enzyme working solution must be use out within 20 min.

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 reagent 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 9 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 9 mL. It can be store at 2-8°C and avoid direct sunlight for 1 month.

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 4°C in the dark.

6. Preparation of **enzyme working solution:** Mix nitrosogenic agent, substrate solution and enzyme stock application solution at a ratio of 1:1:1 fully. Prepare the fresh solution before use and it must be use out within 20 min.

7. Preparation of **non-Enzyme working solution:** Mix nitrosogenic agent, substrate solution and enzyme diluent at a ratio of 1:1:1 fully. Prepare the fresh solution before use and it must be use out within 20 min. (Just need to prepare the solution for 3 wells)

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:

Determine the optimum dilution multiple of the sample before formal experiment. Calculate the inhibition ratio of serial dilution multiple of sample, and choose the optimum dilution multiple when inhibition ratio in the range of 25%~45%.

$$\text{Inhibition ratio} = \frac{\left(A_{control} - A_{Control_{blank}}\right) - \left(A_{sample} - A_{Control_{blank}}\right)}{A_{control} - A_{Control_{blank}}} \times 100\%$$

$$= \frac{A_{control} - A_{sample}}{A_{control} - A_{Control_{blank}}} \times 100\%$$

If inhibition ratio is more than 50%, dilute the sample and then carry the assay. If inhibition ratio is less than 10%, increase the dilution multiple.

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

Sample Type:	Dilution Factor:
Human serum	2-4
Rat serum	4-6
Mouse serum	4-6
HepG2 cell	15-30
Human urine	2-5
10% Mouse liver tissue homogenate	160-200
10% Epipremnum aureumtissue homogenate	3-5
10% Mouse brain tissue homogenate	80-100

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

Plate Set Up:

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

Note: $control_{blank}$ wells; B, control wells; S1-S92, sample wells.

10. Operation Steps

1. **Control blank well:** add 5 μL of PBS (0.01 M, pH 7.4) to the Control blank wells.
   Control well: add 5 μL of PBS (0.01 M, pH 7.4) to the Control wells.
   Sample well: add 5 μL of sample to the Sample wells.

2. Add 90 μL of buffer working solution into each well of Step 1.

3. **Control blank well:** add 30 μL of Non-Enzyme working solution.
   Control well: add 30 μL of Enzyme working solution.
   Sample well: add 30 μL of Enzyme working solution

4. Shake for 10 s with microplate reader and cover the plate with sealer, incubate for 50 min at 37°C.

5. Add 180 μL of chromogenic agent into each well of Step 4.

6. Shake for 10 s with microplate reader and stand for 10 min at room temperature. Measure the OD value of each well at 550 nm with microplate reader

Operation Table

	Sample well	Control well	Control blank well
Sample (μL)	5		
PBS (0.01 M, pH 7.4) (μL)		5	5
Buffer working solution (μL)	90	90	90
Enzyme working solution (μL)	30	30	
Non-Enzyme working solution (μL)			30
Shake for 10 s with microplate reader and cover the plate with sealer, incubate for 50 min at 37°C.			
Chromogenic agent (μL)	180	180	180
Shake for 10 s with microplate reader and stand for 10 min at room temperature. Measure the OD value of each well at 550 nm with microplate reader.			

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 (%), $i = \frac{(A_1-A_3)-(A_2-A_3)}{A_1-A_3} \times 100\% = \frac{A_1-A_2}{A_1-A_3} \times 100\%$

A_1: the OD value of control well at 550 nm

A_2: the OD value of sample well at 550 nm

A_3: the OD value of $Control_{blank}$ well 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

12. Performance Characteristics

Detection Range	2.4-61 U/mL
Sensitivity	2.4 U/mL
Average recovery rate (%)	105
Average inter-assay CV (%)	5.6
Average intra-assay CV (%)	5.5

Analysis

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) for 160 times before use, take 5 μL sample dilution, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample well is 0.248, the average OD value of the control well is 0.333, the average OD value of the $control_{blank}$ well is 0.132, the concentration of protein in sample is 11.61 mgprot/mL, and the calculation result is:

$$\text{T-SOD activity (U/mgprot)} = \left(\frac{0.333 - 0.248}{0.333 - 0.132}\right) \div 50\% \times \frac{0.305}{0.005} \times 160 \div 11.61$$

$$= 711 \text{ U/mgprot}$$

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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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Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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