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

Catalog No: E-BC-K019-M

Method: Colorimetric method

Specification: 96T (Can detect 92 samples without duplication)

Instrument: Microplate reader

Sensitivity: 2.4 U/mL

Detection range: 2.4-61 U/mL

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲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.

▲Background

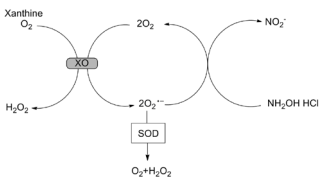
According to the literature, superoxide dismutase exists in all oxygen-metabolizing cells to protect cells from excessive superoxide. Under the action of SOD, two superoxide anions were converted to oxygen and hydrogen peroxide. The reaction principle is as follows:

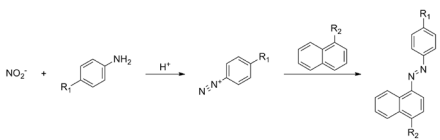
$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

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.

▲Detection principle

The superoxide anion free radical ($O2^{\cdot-}$) can be produced by xanthine and xanthine oxidase reaction system, $O2^{\cdot-}$ 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 ($O2^{\cdot-}$). 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.





▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	1.2 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Nitrosogenic Agent	1.2 mL × 1 vial	2-8℃ , 6 months
Reagent 3	Substrate Solution	1.2 mL × 1 vial	2-8℃ , 6 months
Reagent 4	Enzyme Stock Solution	0.06 mL × 1 vial	-20℃ , 6 months
Reagent 5	Enzyme Diluent	1.2 mL × 1 vial	2-8℃ , 6 months
Reagent 6	Chromogenic Agent A	Powder × 1 vial	2-8℃ , 6 months, shading light
Reagent 7	Chromogenic Agent B	Powder × 1 vial	2-8℃ , 6 months, shading light
Reagent 8	Chromogenic Agent C	6 mL × 1 vial	2-8℃ , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader(540-560 nm), Micropipettor, Centrifuge, Incubator

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key points of the assay

1. 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%.

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

$$= \frac{(A_{control} - A_{sample})}{(A_{control} - A_{control_{blank}})} \times 100\%$$

   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.

2. EDTA should not be as anticoagulation, suggest to use heparin plasma.
3. The prepared enzyme working solution must be use out within 20 min.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 1 working solution:
   Dilute the reagent 1 with double distilled water at a ratio of 1:9 before use. Prepared solution can be stored at 2-8℃ for 3 months.
2. Preparation of reagent 4 working solution:
   Dilute reagent 4 with reagent 5 at a ratio of 1:19. Prepare the fresh solution before use. Unused reagent can be stored at 2-8℃ for 3 days.
3. Preparation of reagent 6 application solution:
   Dissolve a vial of powder with 70-80℃ double distilled water to a final volume of 9 mL. It can be store at 2-8℃ with shading light for 3 months.
4. Preparation of reagent 7 application solution:
   Dissolve a vial of powder with double distilled water to a final volume of 9 mL. It can be store at 2-8℃ with shading light for 1 months.
5. Preparation of chromogenic agent:
   Prepare chromogenic agent at ratio of reagent 6 application solution: reagent 7 application solution: reagent 8 =3:3:2. Prepare the fresh solution before use and the prepared chromogenic agent can be stored at 4℃ in the dark.
6. Preparation of enzyme working solution:
   Mix reagent 2, reagent 3 and reagent 4 working 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 reagent 2, reagent 3 and reagent 5 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)

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

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.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment.

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%.

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

$$= \frac{(A_{control}-A_{sample})}{(A_{control}-A_{control_{blank}})} \times 100\%$$

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).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	550 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

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

▲Operating 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 Reagent 1 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 ℃ .

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.

Note: Control well and $Control_{blank}$ well can be done with 2 wells respectively.

▲Operation table

	Sample well	Control well	$Control_{blank}$ well
Sample (μL)	5		
PBS (0.01 M, pH 7.4) (μL)		5	5
Reagent 1 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 ℃ .			
Chromogenic agent (μL)	180	180	180

▲Calculation

1. For serum (plasma), culture cell and other liquid samples:

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

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

2. For animal tissue and cells 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).

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

Note:

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.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. If the T-SOD activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

Appendix I Performance characteristics

Appendix I Performance characteristics			
Detection range	2.4-61 U/mL	Average intra-assay CV (%)	5.5
Sensitivity	2.4 U/mL	Average inter-assay CV (%)	5.6
Average recovery rate (%)	105		

▲Example 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, then 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 controlblank well is 0.132, the concentration of protein in sample is 11.61 mgprot/mL, and the calculation result is:

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

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . 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℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant), centrifuge at 1000-2000 g for 10 min at 4℃ .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℃ for a month.

▲ Urine

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

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . 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℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . 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℃ for a month.

▲ Cells

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 PBS (0.01 M, pH 7.4) at a ratio of cell number (10^6): PBS (0.01 M, pH 7.4) (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 1500 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℃ for a month.

Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 10 min).

▲ Notes for sample

1. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
2. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.