PRODUCT INFORMATION & MANUAL

Total Superoxide Dismutase/T-SOD Activity Assay Kit (Colorimetric)

NBP3-25884

For research use only.
Not for diagnostic or therapeutic procedures.

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Total Superoxide Dismutase/T-SOD Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25884

Method: Colorimetric method

Specification: 100 Assays (Can detect 50 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 4.7 U/mL

Detection range: 4.7-166 U/mL

Average intra-assay CV (%): 2.8

Average inter-assay CV (%): 6.3

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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.

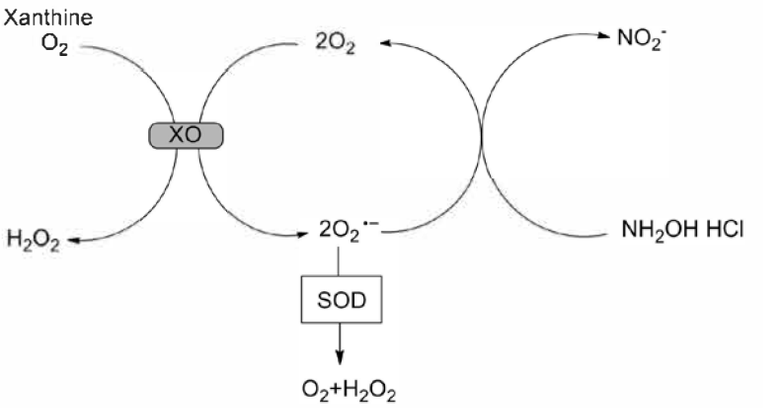
▲Background

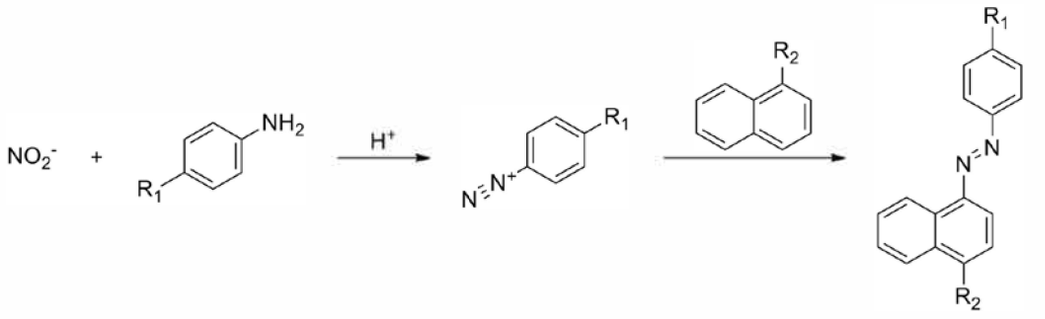
According to the literature, superoxide dismutase exists in all oxygen-metabolizing cells to protect cells from excessive superoxide. Under the action of SOD, two superoxide anions were converted to oxygen and hydrogen peroxide. The reaction principle is as follows:

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 ($O2^{\bullet-}$) can be produced by xanthine and xanthine oxidase reaction system,$O2^{\bullet-}$ 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 ($O2^{\bullet-}$). 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	12 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Nitrosogenic Agent	12 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Substrate Solution	12 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Enzyme Stock Solution	0. 6mL × 1 vial	-20℃ , 12 months
Reagent 5	Enzyme Diluent	12 mL × 1 vial	2-8℃ , 12 months
Reagent 6	Chromogenic Agent A	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 7	Chromogenic Agent B	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 8	Chromogenic Agent C	60 mL × 1 vial	2-8℃ , 12 months
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

Spectrophotometer (550 nm), Micropipettor, Vortex mixer, Water bath, Centrifuge

Reagents

Double distilled water, Normal saline (0. 9%NaCl) or PBS (0. 01M, 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. 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℃ . The temperature (37℃) of incubation should be stable.
2. It is recommended to use heparin as anticoagulant instead of EDTA.
3. The Inhibition ratio of this kit is 15-55%, the optimal inhibition ratio is 25-45%. When the inhibition ratio is 25-45%, the corresponding sampling volume is the optimal sampling volume.

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

If inhibition ratio > 55%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 15%, need to increase the sampling volume.

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 90 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 90 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 2-8℃ in the dark.

▲Sample preparation

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

Sample requirements

The samples should not contain SDS, Tween 20, NP-40, Triton X-100 and other detergents, and should not contain DTT, 2-mercaptoethanol and other reducing reagents

▲Determination of optimal sampling volume

1. 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 to determining optimal sampling volume before formal experiment.
2. The Inhibition ratio of this kit is 15-55%, the optimal inhibition ratio is 25-45%. When the inhibition ratio is 25-45%, the corresponding sampling volume is the optimal sampling volume.

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

If inhibition ratio > 55%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 15%, need to increase the sampling volume.

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).

Assay protocol

▲Detailed operation steps

1. Sample tube: add 1 mL of reagent 1 working solution and a* mL sample to the sample tubes.
   Control tube: add 1 mL of reagent 1 working solution and a* mL double distilled water to the control tubes.
2. Add 0.1 mL of reagent 2, 0.1 mL of reagent 3, 0.1 mL of reagent 4 working solution successively into the tubes of Step 1.
3. Mix fully with a vortex mixer, incubate for 40 min at 37 ℃ .
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.

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

▲Summary operation table

	Sample tube	Control tube
Reagent 1 working solution (mL)	1.0	1.0
Sample (mL)	a*	
Double distilled water(mL)		a*
Reagent 2 (mL)	0.1	0.1
Reagent 3 (mL)	0.1	0.1
Reagent 4 working solution (mL)	0.1	0.1
Mix fully with a vortex mixer, incubate for 40 min at 37 ℃ .		
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.		

▲Calculation

1. For serum (plasma), culture cell supernatant 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, $i = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100\%$

V_1: the total volume of reaction solution, mL.

V_2: the volume of sample added, mL.

f: dilution factor of sample before test.

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

▲Notes

1. It is best to reserve 3 paralleled tubes with different sampling volumes in pre-test for determining the optimal sampling volume. The sampling volume in examples as median, increase by 10 μL and decrease by 10 μL. Take the pre-test with 3 paralleled tubes and 1 control tube to determining the optimal sampling volume.
2. All the reagents should be prepared at the day before the experiment, in order to let the reagents dissolve fully. Please bring all the reagents and samples to room temperature for 30 min before the assay.
3. If the T-SOD activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately .

Appendix I Data

▲Example 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 and 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:

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

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

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 homogenized medium (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 homogenization medium at a ratio of cell number (10^6): homogenization medium (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.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4).
2. 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).

Appendix III References

1. Perry J J, Shin D S, Getzoff E D, et al. The structural biochemistry of the superoxide dismutases[J]. Biochim Biophys Acta, 2010, 1804: 245-262.
2. Miller A F. Superoxide dismutases: ancient enzymes and new insights[J]. FEBS Lett, 2012, 586: 585-595.
3. Cristiana F, Elena A, Nina Z. Superoxide Dismutase: Therapeutic Targets in SOD Related Pathology[J]. Scientific Research, 2014, 06: 975-988.