PRODUCT INFORMATION & MANUAL

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

NBP3-25885

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: NBP3-25885

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.2 U/mL

Detection range: 0.2 -14.4 U/mL

Average intra-assay CV (%): 2.9

Average inter-assay CV (%): 3.7

Average recovery rate (%): 97

- ▲ 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, pleural effusion, ascites, urine, cells, various animal and plant tissues samples.

▲Background

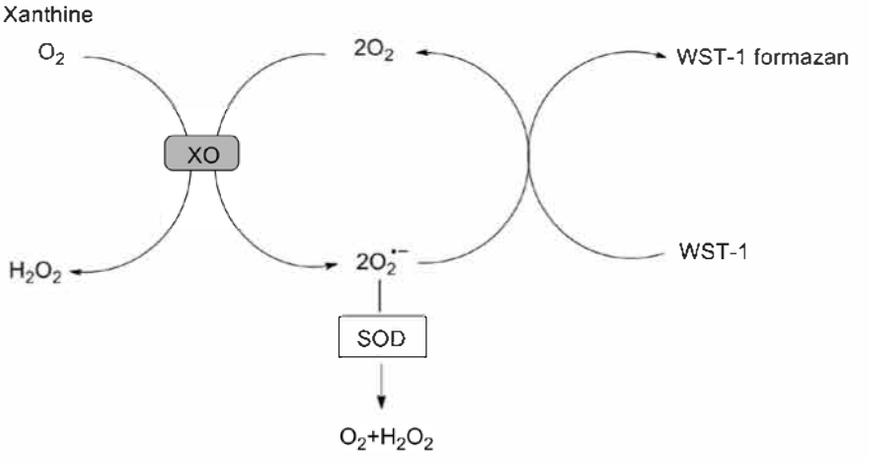
According to the literature, superoxide dismutase exists in all oxygen-metabolizing cells to protect cells from excessive superoxide. Under the action of SOD, two superoxide anions were converted to oxygen and hydrogen peroxide. The reaction principle is as follows:

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

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 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 $O2^{\bullet-}$ 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.



▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	24 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Substrate Solution	0.14 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 3	Enzyme Stock Solution	0.3 mL ×1 vial	-20℃ , 12 months
Reagent 4	Enzyme Diluent	1.5 mL × 2 vials	2-8℃ , 12 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 (440-460 nm), Micropipettor, Multichannel pipettor, Vortex mixer, Incubator

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. 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.

Pre-assay preparation

▲Reagent preparation

1. Preparation of substrate application solution:

Mix the reagent 1 and reagent 2 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℃ for 7 days.

2. Preparation of enzyme working solution:

Mix the reagent 3 and reagent 4 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℃ for 3 days.

Note: Reagent 3 should melt slowly on ice. It is recommended to aliquot the reagent 3 into smaller quantities for optimal storage. Avoid repeated freeze-thaw cycles. Bring all reagents to room temperature before use.

▲Sample preparation

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

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.

▲Dilution of sample

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

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

Assay protocol

▲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;

▲Detailed 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℃ for 20 min. Measure the OD values of each well at 450 nm with microplate reader.

▲Summary 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 and incubate at 37℃ for 20 min. Measure the OD values of each well.			

▲Calculation

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

2. Calculation formula:

$$i=\frac{(A_{control}-A_{blank_{control}})-(A_{sample}-A_{blank_{control}})}{(A_{control}-A_{blank_{control}})}\times 100\%$$

(1) For serum (plasma) sample:

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

(2) For tissue and cells:

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

Note:

i: Inhibition ratio of SOD (%).

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.

Appendix I Data

▲Example analysis

Take human serum, dilute for 4 times with PBS, then take 0.02 mL of diluted sample and 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 BlankControl is 0.048, the average OD value of sample is 0.388, and the calculation result is:

$$i=\frac{(0.608-0.048)-(0.388-0.048)}{(0.608-0.048)}\times 100\% = 39.29\%$$

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

$$= 47.15 \text{ (U/mgprot)}$$

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 recommended), 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 homogenization medium at 2-8℃ to remove blood cells. 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. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). 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. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). 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.