PRODUCT INFORMATION & MANUAL

SOD1/Cu-Zn SOD Activity Assay Kit (Colorimetric)

NBP3-24484

For research use only.

Not for diagnostic or therapeutic procedures.

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SOD1/Cu-Zn SOD Activity Assay Kit (Colorimetric) (Hydroxylamine Method)

Catalog No: NBP3-24484

Method: Colorimetric method

Specification: 96T (Can detect 92 samples for CuZn-SOD only without duplication, or detect 44 samples for both CuZn-SOD and Mn-SOD without duplication)

Measuring instrument: Microplate reader

Sensitivity: 1.35 U/mL

Detection range: 1.35-62 U/mL

Average intra-assay CV (%): 5.1

Average inter-assay CV (%): 9.6

Average recovery rate (%): 99

- ▲ 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 T-SOD, CuZn-SOD, Mn-SOD activity in serum, plasma, urine, cells, cell culture supernatant and tissue homogenate samples.

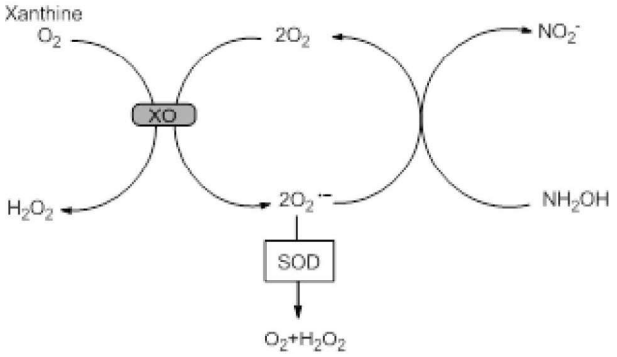
Background

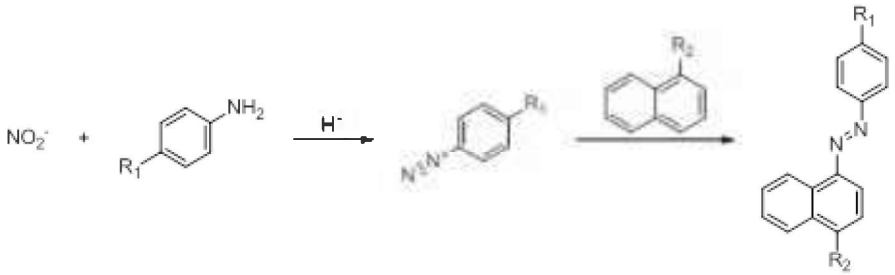
According to the literature, superoxide dismutase exists in all oxygen-metabolizing cells to protect cells from excessive superoxide. Under the action of SOD, two superoxide anions were converted to oxygen and hydrogen peroxide.

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

Superoxide anion ($O2^{\cdot-}$) produced by xanthine and xanthine oxidase system can oxidize hydroxylamine to form nitrite which appear purplish red after chromogenic reaction. The SOD in the sample has a specific inhibitory effect on superoxide anion ($O2^{\cdot-}$), can reduce the content of nitrite. The OD value is lower than control and the activity of SOD is calculated through the formula. There are two kinds of SOD (CuZn-SOD, Mn-SOD) in the cells of higher animals, and the sum of them is equal to the total SOD. The activity of Mn-SOD in the sample will lost after sample pretreatment, but the activity of CuZn-SOD will not.





▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	1.2 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Nitrosogenic Agent	1.2 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Substrate Solution	1.2 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Enzyme Stock Solution	0.06 mL × 1 vial	-20℃ , 12 months
Reagent 5	Enzyme Diluent	1.2 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	6 mL × 1 vial	2-8℃ , 12 months
Reagent 9	Extracting Solution	12 mL × 1 vial	2-8℃ , 12 months, shading light
	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 (530-570 nm), Micropipettor, Centrifuge, Incubator,

Vortex mixer

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲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 optimal sampling volume of each sample before formal experiment. Calculate the inhibition ratio of serial sampling volume, and choose the optimal sampling volume when inhibition ratio in the range of 25%~45%.
2. The optimal sampling volume are different for different species, the SOD also are different for different samples. So it is best to do a pre-test to determining optimal sampling volume for a new sample.
3. It is best to reserve 3 paralleled tubes with different sampling volumes in pre-test for determining the optimal sampling volume.
4. Adjust sampling volume: 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.
5. There should be no bubbles in the wells of the microplate when measuring the OD value.
6. The multichannel pipeter is recommended to shorten the time and reduce the error between wells.

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:

(Operate on ice) 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 stored 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 2-8℃ in the dark.

6. Preparation of enzyme working solution:

(Operate on ice) Mix the reagent 2, reagent 3 and reagent 4 working solution at the ratio of 1: 1: 1. Prepare the fresh solution before use and prepared solution should be used in 20 min.

7. Preparation of non-enzyme working solution:

Mix the reagent 2, reagent 3 and reagent 5 at the ratio of 1: 1: 1. Prepare the fresh needed amount before use and prepared solution should be used in 20 min.

▲Sample preparation

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

Sample requirements

1. Samples should not contain decontamination agents such as SDS, Tween-20, NP-40, Triton X-100, nor reductive reagents such as DTT, 2- mercaptoethanol.
2. EDTA should not be as anticoagulation, suggest to use heparin plasma.

▲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 15%~55% (the optimal inhibition ratio is the range of 25%~45%.) before formal experiment.

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

Sample type	Dilution factor
Mouse serum	5-10
Rat serum	6-15
Urine	2-3
Human hydrothorax	2-3
10% Mouse liver tissue homogenate	100-200
10% Mouse brain tissue homogenate	20-30
10% Mouse kidney tissue homogenate	50-120
10% Rat kidney tissue homogenate	50-120
HepG2 cells (5.21mgprot/mL)	15-25
Cell culture supernatant	1

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

1. The detection for CuZn-SOD only

	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, the blank well of CuZn-SOD; B, the control well of CuZn-SOD;
S1-S92, sample wells.

2. The detection for CuZn-SOD and Mn-SOD

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	C	C	S13'	S21'	S29'	S37'
B	B	B	S14	S22	S30	S38	D	D	S14'	S22'	S30'	S38'
C	S1	S7	S15	S23	S31	S39	S1'	S7'	S15'	S23'	S31'	S39'
D	S2	S8	S16	S24	S32	S40	S2'	S8'	S16'	S24'	S32'	S40'
E	S3	S9	S17	S25	S33	S41	S3'	S9'	S17'	S25'	S33'	S41'
F	S4	S10	S18	S26	S34	S42	S4'	S10'	S18'	S26'	S34'	S42'
G	S5	S11	S19	S27	S35	S43	S5'	S11'	S19'	S27'	S35'	S43'
H	S6	S12	S20	S28	S36	S44	S6'	S12'	S20'	S28'	S36'	S44'

Note: A, the blank well of T-SOD; B, the control well of T-SOD; S1-S44, the sample wells of T-SOD; C, the blank well of CuZn-SOD; D, the control well of CuZn-SOD; S1'-S44', the sample wells of CuZn-SOD.

▲Operating steps

1. Sample pretreatment

1) Take 0.1 mL sample and add 0.1 mL reagent 9. Mix thoroughly with a vortex mixer for 1 min by vortex mixer and centrifuge at 3500 g for 15 min. Take supernatant for CuZn-SOD measurement.

2) Take 0.1 mL normal saline and add 0.1 mL reagent 9. Mix thoroughly for 1 min with a vortex mixer and centrifuge at 3500 g for 15 min. Take supernatant as control of CuZn-SOD.

2. The measurement of samples

1) Blank well of T-SOD: take 5 μL of double distilled water to the corresponding wells.

Control well of T-SOD: take 5 μL of double distilled water to the corresponding wells.

Sample well of T-SOD: take 5 μL of sample to the corresponding wells.

Blank well of CuZn-SOD: take 5 μL of supernatant as control of CuZn-SOD to the corresponding wells.

Control well of CuZn-SOD: take 5 μL of supernatant as control of CuZn-SOD to the corresponding wells.

Sample well of CuZn-SOD: take 5 μL of supernatant for CuZn-SOD to the corresponding wells.

2) Add 90 μL of reagent 1 working solution to each well.

3) Add 30 μL of enzyme working solution to control well of T-SOD, sample well of T-SOD, control well of CuZn-SOD, sample well of CuZn-SOD.

Add 30 μL of non-enzyme working solution to blank well of T-SOD and blank well of CuZn-SOD.

4) Mix fully for 10 s with microplate reader and incubate for 50 min at 37℃ .

5) Add 180 μL of chromogenic agent to each well.

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

▲Operation table

	T-SOD			CuZn-SOD		
	Sample tube	Control tube	Blank tube	Sample tube	Control tube	Blank tube
Sample (μL)	5					
Double distilled water (μL)		5	5			
Supernatant for CuZn-SOD (μL)				5		
Supernatant as control of CuZn-SOD (μL)					5	5
Reagent 1 working solution (μL)	90	90	90	90	90	90
Enzyme working solution (μL)	30	30		30	30	
Non-enzyme working solution (μL)			30			30
Mix fully for 10 s with microplate reader and incubate for 50 min at 37℃ .						
Chromogenic agent (μL)	180	180	180	180	180	180
Mix fully for 10 s with microplate reader and stand for 10 min at room temperature. Measure the OD value of each well at 550 nm.						

▲Calculation

1. Calculation formula of SOD activity in serum (plasma), cell culture medium and other liquid samples

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

$$\text{T-SOD activity (U/mL)} = i_1 \div 50\% \times \frac{V_1}{V_2} \times f$$

$$\text{CuZn-SOD activity (U/mL)} = i_2 \div 50\% \times \frac{V_1}{V_2} \times f$$

2. Calculation formula of SOD activity in tissue and cell samples

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

$$\text{T-SOD activity (U/mL)} = i_1 \div 50\% \times \frac{V_1}{V_2} \times f \div C_{pr}$$

$$\text{CuZn-SOD activity (U/mL)} = i_2 \div 50\% \times \frac{V_1}{V_2} \times f \div C_{pr}$$

$$\text{Mn-SOD activity} = \text{T-SOD activity} - \text{CuZn-SOD activity}$$

Note:

i_1: Inhibition ratio of T-SOD

$$i_1(\%) = \frac{(A_1-A_3) - (A_2-A_3)}{A_1-A_3} \times 100\% = \frac{(A_1-A_2)}{(A_1-A_3)} \times 100\%$$

i_2: Inhibition ratio of CuZn-SOD

$$i_2(\%) = \frac{(A_4-A_6) - (A_5-A_6)}{A_4-A_6} \times 100\% = \frac{(A_4-A_5)}{(A_4-A_6)} \times 100\%$$

A_1: The OD value of $T\text{-}SOD_{Control}$

A_2: The OD value of $T\text{-}SOD_{Sample}$

A_3: The OD value of $T\text{-}SOD_{Blank}$

A_4: The OD value of $CuZn\text{-}SOD_{Control}$

A_5: The OD value of $CuZn\text{-}SOD_{Sample}$

A_6: The OD value of $CuZn\text{-}SOD_{Blank}$

V_1: The total volume of the reaction system (mL)

V_2: The volume of sample added to the reaction system (mL)

C_{pr} Protein concentration of sample (mgprot/mL)

f: Dilution factor of sample before tested

Appendix I Data

▲Example analysis

The detection of T-SOD: Take 10% mouse liver tissue homogenate, dilute for 150 times with normal saline (0.9% NaCl), then take 5 μL of diluted sample and carry the assay according to the operation table. The results are as follows: The average OD value of $T\text{-}SOD_{Sample}$ is 0.344, the average OD value of $T\text{-}SOD_{Control}$ is 0.546, the average OD value of $T\text{-}SOD_{Blank}$ is 0.121, the concentration of protein in 10% mouse liver tissue homogenate is 13.72 mgprot/mL, and the calculation result is:

$$\text{T-SOD activity (U/mgprot)} = \frac{0.546-0.344}{0.546-0.121} \div 50\% \times \frac{0.305}{0.005} \times 150 \div 13.72 = 633.96 \text{ U/mgprot}$$

The detection of CuZn-SOD: pretreat the diluted sample with reagent 9 and carry the assay according to the operation table. The results are as follows:

The average OD value of $CuZn\text{-}SOD_{Sample}$ is 0.400, the average OD value of $CuZn\text{-}SOD_{Control}$ is 0.590, the average OD value of $CuZn\text{-}SOD_{Blank}$ is 0.124, the concentration of protein in 10% mouse liver tissue homogenate is 13.72 mgprot/mL, and the calculation result is:

$$\text{CuZn-SOD activity (U/mgprot)} = \frac{0.590-0.400}{0.590-0.124} \div 50\% \times \frac{0.305}{0.005} \times 150 \div 13.72 = 543.83 \text{ U/mgprot}$$

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 (do not use EDTA as anticoagulation, 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 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. 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: Normal saline (0.9% NaCl).

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.