（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K020-M

Specification：48T(44 samples)/96T(92 samples)/ 500Assays (496 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.2 -14.4 U/mL

Elabscience® Total Superoxide Dismutase (T-SOD) Activity Assay Kit (WST-1 Method)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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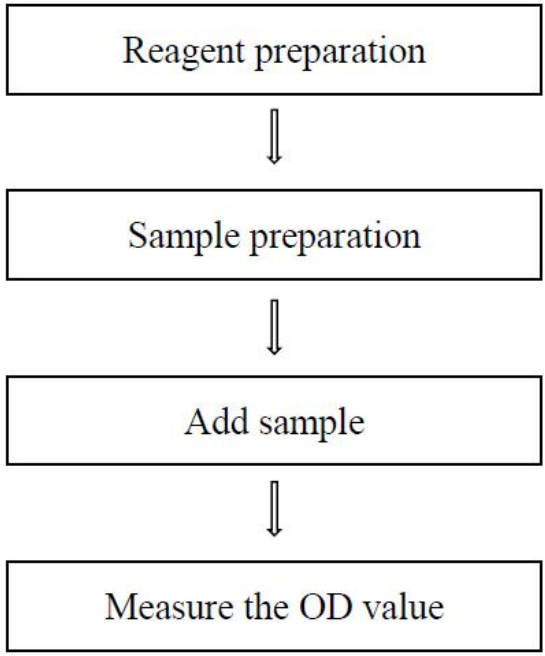
Assay summary



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.

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•- 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	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Buffer Solution	12 mL×1 vial	24 mL×1 vial	60 mL×2 vials	2-8℃, 12 months
Reagent 2	Substrate Solution	0.07 mL×1 vial	0.14 mL×1 vial	1 mL×1 vial	2-8℃, 12 months shading light
Reagent 3	Enzyme Stock Solution	0.15 mL×1 vial	0.3 mL×1 vial	1.5 mL×1 vial	-20℃, 12 months
Reagent 4	Enzyme Diluent	1.5 mL×1 vial	1.5 mL×2 vials	15 mL×1 vial	2-8℃, 12 months
	Microplate	48 wells	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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)

Reagent preparation

① Keep enzyme stock solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of substrate application solution：

Before testing, please prepare sufficient substrate application solution according to the test wells. For example, prepare 1005 μL of substrate application solution (mix well 1000 μL of buffer solution and 5 μL of substrate solution). The substrate application solution should be prepared on spot. Store at 2-8°C for 7 days.

③ The preparation of enzyme working solution：

Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 220 μL of enzyme working solution (mix well 20 μL of enzyme stock solution and 200 μL of enzyme diluent). (Note: Please operate on the ice box.) The enzyme working solution should be prepared on spot. Store at 2-8°C for 3 days.

Sample preparation

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.

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Urine: Collect fresh urine and centrifuge at 10000×g for 15 min to remove insoluble material. Collect supernatant and keep it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 10^6 cells in 300-500 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② 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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

① Control well: add 20 μL of double distilled water and 20 μL of enzyme working solution into the control wells.

$Blank_{Control}$ well: add 20 μL of double distilled water and 20 μL of enzyme diluent into the $blank_{control}$ wells.

Sample well: add 20 μL of sample and 20 μL of enzyme working solution into the sample wells.

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.

Calculation

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

$$i = \frac{(A_{Control}-A_{Blank_{Control}})-(A_{Sample}-A_{Blank_{Control}})}{A_{Control}-A_{Blank_{Control}}} \times 100\%$$

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample and cells sample:

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

[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 test.

C_{pr}: Concentration of protein in sample, gprot/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	1.20	8.40	12.50
%CV	3.2	3.0	2.5

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	1.20	8.40	12.50
%CV	3.4	3.8	3.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	3.5	9.6	13
Observed Conc. (U/mL)	3.5	9.1	12.6
Recovery rate (%)	99	95	97

Sensitivity

The analytical sensitivity of the assay is 0.2 U/mL T-SOD. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

Example analysis：

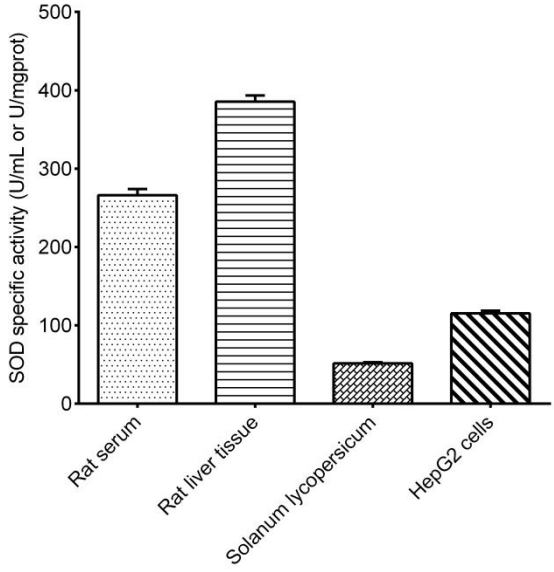
Take human serum, dilute for 5 times with PBS, then take 0.02 mL of diluted sample, and carry the assay according to the operation steps. The results are as follows:

the average OD value of the control is 0.608, the average OD value of $blank_{control}$ is 0.048, the average OD value of sample is 0.388, and the calculation result is:

$$\text{Inhibition ratio of SOD (\%)} = \frac{(0.608-0.048)-(0.388-0.048)}{(0.608-0.048)} \times 100\% = 39.29\%$$

$$\text{SOD activity (U/mgprot)} = 39.29\% \div 50\% \times \frac{0.24}{0.02} \times 5 = 47.15\ (\text{U/mgprot})$$

Detect rat serum (dilute for 20 times), 10% rat liver tissue homogenate (the concentration of protein is 10.67 mgprot/mL, dilute for 360 times), 20% tomato tissue homogenate (the concentration of protein is 2.44 mgprot/mL, dilute for 10 times) and HepG2 cells (the concentration of protein is 3.18 mgprot/mL, dilute for 30 times) according to the protocol, the result is as follows：



Appendix III Publications

1. Li M , Liu J , Shi L ,et al.Gold nanoparticles-embedded ceria with enhanced antioxidant activities for treating inflammatory bowel disease[J], 2023.DOI:10.1016/j.bioactmat.2023.01.015.
2. Wang Y, Liang X, Andrikopoulos N, et al. Remediation of Metal Oxide Nanotoxicity with a Functional Amyloid[J]. Advanced Science, 2024, 11(23): 2310314.
3. Wang X, Wang J, Liu S, et al. Sterilization mechanism and nanotoxicity of visible light-driven defective carbon nitride and UV-excited TiO2[J]. Journal of Hazardous Materials, 2024, 461: 132109.
4. Wang J, Pu X, Zhuang H, et al. Astragaloside IV alleviates septic myocardial injury through DUSP1-Prohibitin 2 mediated mitochondrial quality control and ER-autophagy[J]. Journal of Advanced Research, 2024.
5. Xiao R , Liu J , Shi L ,et al.Au-modified ceria nanozyme prevents and treats hypoxia-induced pulmonary hypertension with greatly improved enzymatic activity and safety[J].Journal of nanobiotechnology, 22(1):492[2025-03-03].DOI:10.1186/s12951-024-02738-4.
6. Wan Q , Cao R , Wen G ,et al.Sequential use of UV-LEDs irradiation and chlorine to disinfect waterborne fungal spores: Efficiency, mechanism and photoreactivation[J].Journal of Hazardous Materials, 2022, 423:127102-.DOI:10.1016/j.jhazmat.2021.127102.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.