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

Catalog No：E-BC-K019-S

Specification：50 Assays (25 samples)/ 100 Assays (50 samples)

Measuring instrument：Spectrophotometer（550 nm）

Detection range：4.7-166 U/mL

Elabscience® Total Superoxide Dismutase (T-SOD) Activity Assay Kit (Hydroxylamine 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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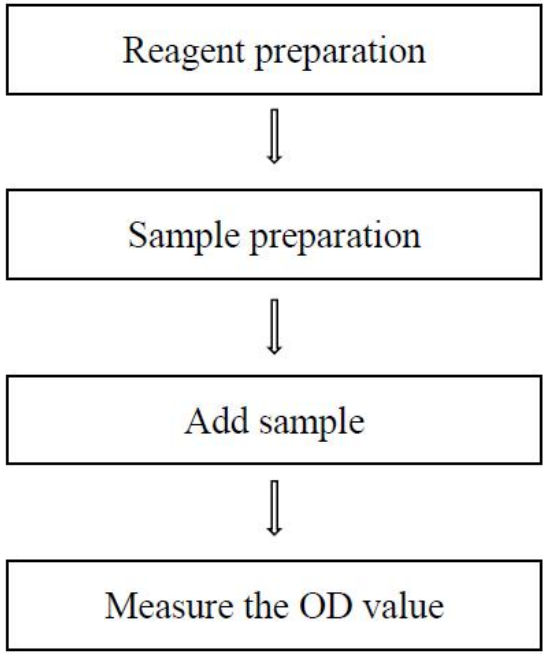
Assay summary



Intended use

This kit can be used to measure total superoxide dismutase (T-SOD) activity in serum, plasma, urine, cells, cell culture supernatant and tissue samples.

Detection principle

The superoxide anion free radical (O2•-) can be produced by xanthine and xanthine oxidase reaction system, O2•- 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•-). 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	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Buffer Solution	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 2	Nitrosogenic Agent	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 3	Substrate Solution	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 4	Enzyme Stock Solution	0.3 mL × 1 vial	0.6 mL × 1 vial	-20℃, 12 months
Reagent 5	Enzyme Diluent	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 6	Chromogenic Agent A	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
Reagent 7	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
Reagent 8	Chromogenic Agent C	30 mL × 1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

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 (0.01 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 buffer working solution:
For each tube, prepare 1.0 mL of buffer working solution (mix well 100 uL of buffer solution and 900 uL of double distilled water). Store at 2-8°C for 3 months.

③ The preparation of enzyme stock working solution:
For each tube, prepare 100 uL of enzyme stock working solution (mix well 5 uL of enzyme stock solution and 95 uL of enzyme diluent). Store at 2-8°C for 3 days.

④ The preparation of chromogenic agent A application solution:
Dissolve one vial of chromogenic agent A with 70-80°C double distilled water to a final volume of 90 mL. Store at 2-8°C for 3 months protected from light.

⑤ The preparation of chromogenic agent B application solution:
Dissolve one vial of chromogenic agent B with double distilled water to a final volume of 90 mL. Store at 2-8°C for 1 month protected from light.

⑥ The preparation of chromogenic agent:
For each tube, prepare 2.0 mL of chromogenic agent (mix well 750 uL of

chromogenic agent A application solution, 750 uL of chromogenic agent B application solution and 500 uL of chromogenic agent C). The chromogenic agent should be prepared on spot. Keep it at 4℃ protected from light during use.

Sample preparation

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 minutes 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℃.

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).

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 1500×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 Inhibition ratio of this kit is 15-55%, the optimal inhibition ratio is 25-45%. When the inhibition ratio is 30-40%, the corresponding sampling volume is the optimal sampling volume.

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

If inhibition ratio > 50%, 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 (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 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 30-40%, the corresponding sampling volume is the optimal sampling volume.

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If inhibition ratio > 50%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 15%, need to increase the sampling volume.

Operating steps

① Sample tube: add 1 mL of buffer working solution and a* mL sample to the sample tubes.

Control tube: add 1 mL of buffer working solution and a* mL double distilled water to the control tubes.

② Add 0.1 mL of nitrosogenic agent, 0.1 mL of substrate solution, 0.1 mL of enzyme stock working solution successively into each tube.

③ Mix fully with a vortex mixer, incubate at 37 ℃ for 40 min.

④ Add 2 mL of chromogenic agent into each tube.

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.

Calculation

The sample:

1. Serum (plasma) sample, 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. Tissue sample 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).

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

[Note]

i: inhibition ratio, $\text{Inhibition ratio} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100\%$.

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}: 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)	15.00	78.00	124.00
%CV	3.2	2.7	2.5

Intra-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)	15.00	78.00	124.00
%CV	5.9	6.7	6.3

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 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	26	106	135
Observed Conc. (U/mL)	28.1	110.2	139.1
Recovery rate (%)	108	104	103

Sensitivity

The analytical sensitivity of the assay is 4.7 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

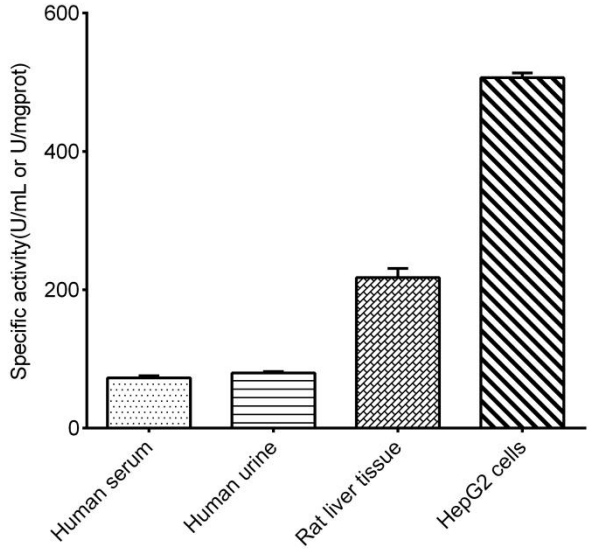
Example analysis：

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) for 10 times before use, take 10 μL sample dilution, and carry the assay according to the operation steps. 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:

$$\text{T-SOD activity (U/mgprot)} = \left(\frac{0.343-0.212}{0.343}\right) \div 50\% \times \frac{3.31}{0.01} \times 10 \div 11.61 = 217.77 \text{ U/mgprot}$$

Detect human serum (V_2=30 μL), human urine (V_2=30 μL), 10% rat liver tissue homogenate (the concentration of protein is 11.61 mgprot/mL, dilute for 10 times, V_2=10 μL), HepG2 cells (the concentration of protein is 3.18 mgprot/mL, dilute for 4 times, V_2=5 μL) according to the protocol, the result is as follows：



Appendix III Publications

1. Liu Y, Wang L, Liu Z, et al. Durable immunomodulatory nanofiber niche for the functional remodeling of cardiovascular tissue[J]. ACS nano, 2023, 18(1): 951-971.
2. Zhang H, Feng Y, Si Y, et al. Shank3 ameliorates neuronal injury after cerebral ischemia/reperfusion via inhibiting oxidative stress and inflammation[J]. Redox Biology, 2024, 69: 102983.
3. Liu H, Ji M, Bi Y, et al. Integration of MyD88 inhibitor into mesoporous cerium oxide nanozymes-based targeted delivery platform for enhancing treatment of ulcerative colitis[J]. Journal of Controlled Release, 2023, 361: 493-509.
4. 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, 2024, 22(1): 492.
5. Wang Z, Yan C, Wang X, et al. Double-edged sword effects of sulfate reduction process in sulfur autotrophic denitrification system: Accelerating nitrogen removal and promoting antibiotic resistance genes spread[J]. Bioresource Technology, 2024, 409: 131239.
6. Liu D, Zhan J, Wang S, et al. Chrysanthemum morifolium attenuates metabolic and alcohol-associated liver disease via gut microbiota and PPARα/γ activation[J]. Phytomedicine, 2024, 130: 155774.

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.