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

Catalog No：E-BC-K019-M

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

Measuring instrument：Microplate reader（540-560 nm）

Detection range：2.4-61 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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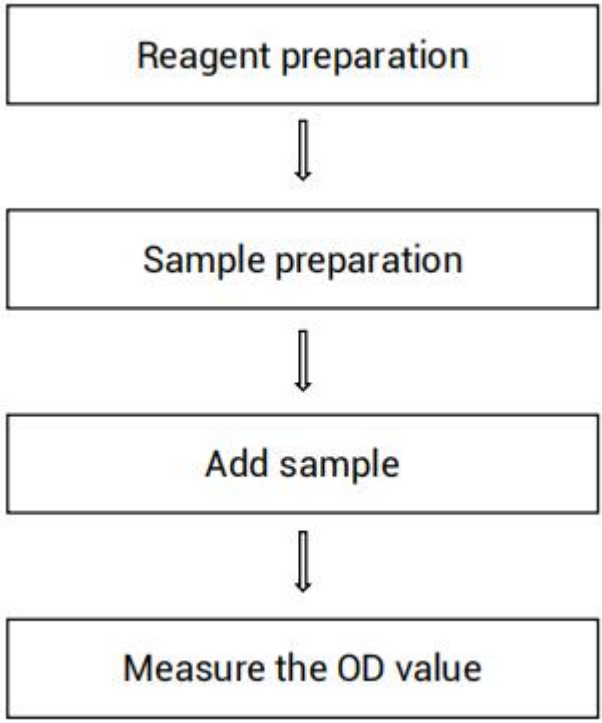
Assay summary



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(48 T)	Size 2(96 T)	Size3 (500Assays)	Storage
Reagent 1	Buffer Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8℃, 12 months
Reagent 2	Nitrosogenic Agent	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8℃, 12 months
Reagent 3	Substrate Solution	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8℃, 12 months
Reagent 4	Enzyme Stock Solution	0.03 mL × 1 vial	0.06 mL × 1 vial	0.06 mL × 5 vials	-20℃, 12 months
Reagent 5	Enzyme Diluent	1.2 mL × 1 vial	1.2 mL × 1 vial	1.2 mL × 5 vials	2-8℃, 12 months
Reagent 6	Chromogenic Agent A	Powder ×1 vial	Powder × 1 vial	Powder × 5 vials	2-8℃, 12 months shading light
Reagent 7	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	Powder × 5 vials	2-8℃, 12 months shading light

Reagent 8	Chromogenic Agent C	6 mL × 1 vial	6 mL × 1 vial	6 mL × 5 vials	2-8℃, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			
	Sample Layout Sheet	1 piece			

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 (520-550 nm, optimum wavelength: 530 nm), Micropipettor, Centrifuge, 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 buffer working solution：

For each well, prepare 90 μL of buffer working solution (mix well 9 μL of buffer solution and 81 μL of double distilled water). Store at 2-8°C for 3 months.

③ The preparation of enzyme stock working solution：

For each well, prepare 40 μL of enzyme stock working solution (mix well 2 μL of enzyme stock solution and 38 μL 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℃ double distilled water to a final volume of 9 mL. Store at 2-8℃ 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 9 mL. Store at 2-8℃ for 1 month protected from light.
⑥ The preparation of chromogenic agent：
For each well, prepare 180 μL of chromogenic agent (mix well 67.5 μL of chromogenic agent A application solution, 67.5μL of chromogenic agent B application solution and 45 μL of chromogenic agent C). The chromogenic agent should be prepared on spot. Keep it at 4℃ protected from light during use.
⑦ The preparation of enzyme working solution：
For each well, prepare 30 μL of enzyme working solution (mix well 10 μL of nitrosogenic agent, 10 μL of substrate solution and 10 μL of enzyme stock working solution). The enzyme working solution should be prepared on spot, and it must be use out within 20 min.
⑧ The preparation of non-enzyme working solution:
For each well, prepare 30 μL of non-enzyme working solution (mix well 10 μL of nitrosogenic agent, 10 μL of substrate solution and 10 μL of enzyme diluent). Store at 2-8℃ for 1 month protected from light. The non-enzyme working solution should be prepared on spot, and it must be use out within 20 min. (Just need to prepare the solution for 3 wells)

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°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.

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.

② Dilution of sample

Determine the optimum dilution multiple of the sample before formal experiment. Calculate the inhibition ratio of serial dilution multiple of sample, and choose the optimum dilution multiple when inhibition ratio in the range of 25%~45%.

$$\text{Inhibition ratio} = \frac{\left(\mathrm{A_{control}} - \mathrm{A_{Control_{blank}}}\right) - \left(\mathrm{A_{sample}} - \mathrm{A_{Control_{blank}}}\right)}{\mathrm{A_{control}} - \mathrm{A_{Control_{blank}}}} \times 100\%$$

$$= \frac{A_{control} - A_{sample}}{\mathrm{A_{control}} - \mathrm{A_{Control_{blank}}}} \times 100\%$$

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

Sample type	Dilution factor
Human serum	2-4
Rat serum	4-6
Mouse serum	4-6
HepG2 cell	15-30
Human urine	2-5
10% Mouse liver tissue homogenate	160-200
10% Epipremnum aureumtissue homogenate	3-5
10% Mouse brain tissue homogenate	80-100

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 Determine the optimum dilution multiple of the sample before formal experiment. Calculate the inhibition ratio of serial dilution multiple of sample, and choose the optimum dilution multiple when inhibition ratio in the range of 25%~45%.

$$\text{Inhibition ratio} = \frac{\left(\mathrm{A_{control}} - \mathrm{A_{Control_{blank}}}\right) - \left(\mathrm{A_{sample}} - \mathrm{A_{Control_{blank}}}\right)}{\mathrm{A_{control}} - \mathrm{A_{Control_{blank}}}} \times 100\%$$

$$= \frac{A_{control} - A_{sample}}{\mathrm{A_{control}} - \mathrm{A_{Control_{blank}}}} \times 100\%$$

If inhibition ratio is more than 50%, dilute the sample and then carry the assay.

If inhibition ratio is less than 10%, increase the dilution multiple.

② EDTA should not be as anticoagulation, suggest to use heparin plasma.

③ The prepared enzyme working solution must be use out within 20 min.

Operating steps

① $Control_{blank}$ well: add 5 μL of PBS (0.01 M, pH 7.4) into the $Control_{blank}$ wells.

Sample well: add 5 μL of sample into the sample wells.

Control well: add 5 μL of PBS (0.01 M, pH 7.4) into the Control wells.

② Add 90 μL of buffer working solution into each well.

③ Add 30 μL of enzyme working solution to the control wells and sample wells. Add 30 μL of non-enzyme working solution to the $control_{blank}$ wells.

④ Mix fully for 10 s with microplate reader and cover the plate with sealer. Incubate at 37℃ for 50 min.

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 values of each well at 550 nm with microplate reader.

Calculation

The sample:

1. Serum (plasma) sample, culture cell 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).

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

[Note]

i: Inhibition ratio of SOD (%), $i = \frac{(A_1\text{-}A_3)\text{-}(A_2\text{-}A_3)}{A_1\text{-}A_3} \times 100\% = \frac{A_1\text{-}A_2}{A_1\text{-}A_3} \times 100\%$

A_1: the OD value of control well at 550 nm.

A_2: the OD value of sample well at 550 nm.

A_3: the OD value of $Control_{blank}$ well at 550 nm.

V_1: the total volume of reaction solution, mL.

V_2: the volume of sample added into the reaction system, 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)	3.50	25.00	45.00
%CV	5.9	5.4	5.2

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)	3.50	25.00	45.00
%CV	5.5	5.3	6.0

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)	15	34	55
Observed Conc. (U/mL)	16.1	36.0	56.1
Recovery rate (%)	107	106	102

Sensitivity

The analytical sensitivity of the assay is 2.4 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 Π Example Analysis

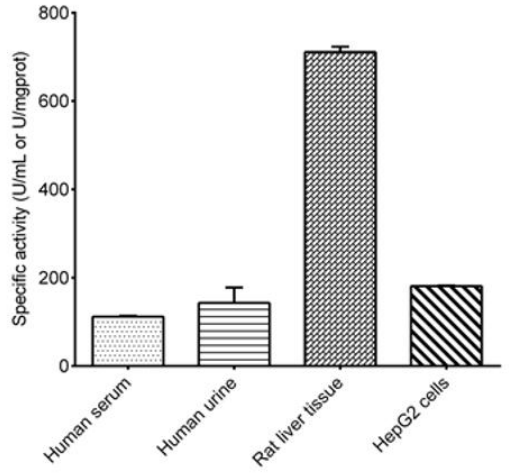
Example analysis：

Dilute 10% rat liver tissue homogenate with PBS (0.01 M, pH 7.4) for 160 times before use, take 5 μL sample dilution, and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample well is 0.248, the average OD value of the control well is 0.333, the average OD value of the controlblank well is 0.132, the concentration of protein in sample is 11.61 mgprot/mL, and the calculation result is:

$$\text{T-SOD activity (U/mgprot)} = \left(\frac{0.333 - 0.248}{0.333 - 0.132}\right) \div 50\% \times \frac{0.305}{0.005} \times 160 \div 11.61 = 711\ \text{U/mgprot}$$

Detect human serum (the concentration of protein in sample, dilute for 2 times, V=5 μL), human urine, (the concentration of protein in sample, dilute for 3 times, V=5 μL), rat liver tissue homogenate (the concentration of protein is 11.61 mgprot/mL, dilute for 160 times, V=5 μL), HepG2 cells (the concentration of protein is 6.09 mg/mL, dilute for 20 times, V=5 μL) according to the protocol, the result is as follows：



Appendix III Publications

1. Zhu N, Chen S, Jin Y, et al. Enhancing Glioblastoma Immunotherapy with Integrated Chimeric Antigen Receptor T Cells through the Re-Education of Tumor-Associated Microglia and Macrophages[J]. ACS nano, 2024, 18(17): 11165-11182.
2. Mo W, Liu S, Zhao X, et al. ROS scavenging nanozyme modulates immunosuppression for sensitized cancer immunotherapy[J]. Advanced Healthcare Materials, 2023, 12(21): 2300191.
3. Yuan J, Ding L, Han L, et al. Thermal/ultrasound-triggered release of liposomes loaded with Ganoderma applanatum polysaccharide from microbubbles for enhanced tumour ablation[J]. Journal of Controlled Release, 2023, 363: 84-100.
4. Wang Y, Kong B, Chen X, et al. BMSC exosome-enriched acellular fish scale scaffolds promote bone regeneration[J]. Journal of Nanobiotechnology, 2022, 20(1): 444.
5. Zeng Z, Quan C, Zhou S, et al. Gut microbiota and metabolic modulation by supplementation of polysaccharide-producing Bacillus licheniformis from Tibetan Yaks: A comprehensive multi-omics analysis[J]. International Journal of Biological Macromolecules, 2024, 254: 127808.
6. Qu B, Zeng Z, Yang H, et al. Resveratrol reversed rosiglitazone administration induced bone loss in rats with type 2 diabetes mellitus[J]. Biomedicine & Pharmacotherapy, 2024, 178: 117208.

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.