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

Catalog No：E-BC-K802-M

Specification：48T(32 samples)/96T(80 samples)/500Assays(484 samples)

Measuring instrument：Microplate reader（580-590 nm）

Detection range：2.5-100 μmol H_2O_2 Equiv./L

Elabscience® Total Oxidant Status (TOS) Colorimetric Assay Kit

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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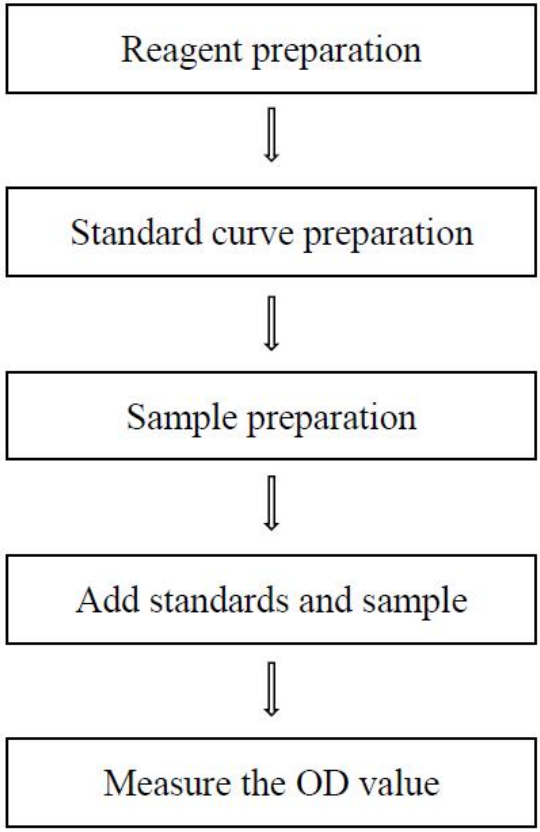
Assay summary



Intended use

The kit is used for the determination of Total Oxidant Status (TOS) in cells，tissue, serum and other liquid samples.

Detection principle

Under acid conditions, the oxidizing material in the sample can oxidize Fe^{2+} to Fe^{3+}, which binds highly with xylenol orange to produce a blue-purple complex. When the pH of solution is in the range of 2-3, its maximum absorption wavelength is around 590 nm, and the color depth is proportional to the content of oxidation substances in a certain concentration and a certain time, so as to indirectly calculate the total oxidation state of the sample.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	Chromogenic Agent	12 mL×1 vial	24 mL×1 vial	60 mL×2 vials	2-8℃, 12 months, shading light
Reagent 2	Substrate	3 mL × 1 vial	6 mL × 1 vial	30 mL× 1 vial	2-8℃, 12 months, shading light
Reagent 3	200 μmol/L H_2O_2 Standard	1 mL × 1 vial	1 mL × 1 vial	5 mL × 1 vial	2-8℃, 12 months, shading light
	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 (580-590 nm, optimum wavelength: 590 nm), Micropipettor, 37℃ incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 200 μmol/L H_2O_2 standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
200 μmol/L H_2O_2 standard (μL)	0	10	20	40	50	60	80	100
Double distilled water (μL)	200	190	180	160	150	140	120	100

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.

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 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).

Cell (adherent or suspension) samples:

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

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

③ Homogenize 1×10^6 cells in 200 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 recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Porcine serum	1
Horse serum	1
Cynomolgus macaques serum	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
0.612×10^6 Molt-4 cells	1

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 It is recommended to aliquot chromogenic agent into smaller quantities in EP tube before use to avoid contamination.

② Substrate should be sealed in time after use and should not be exposed to air for a long time.

③ Avoid bubbles when adding samples.

④ It's better to measure no more than 30 sample wells at same time.

Operating steps

① Standard well: Add 20 μL of standard with different concentration to the standard well.

Sample well: Add 20 μL of sample to the sample well.

② Add 200 μL of chromogenic agent to each well.

③ Mix fully with microplate reader for 5 s and measure the OD values of each well at 590 nm with microplate reader, recorded as A_1.

④ Add 50 μL of substrate to each well.

⑤ Mix fully with microplate reader for 5 s and incubate at 37°C for 5 min. Measure the OD values of each well at 590 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean ΔA value of the blank (Standard #①) from all standard readings. This is the absoluted ΔA value.

3. Plot the standard curve by using absoluted ΔA value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Liquid sample:

$$TOS\ (\mu mol\ H_2O_2\ Equiv./L) = (\Delta A_{590} - b) \div a \times f$$

2. Tissue and cells sample:

$$TOS\ (\mu mol\ H_2O_2\ Equiv./gprot) = (\Delta A_{590} - b) \div a \div C_{pr} \times f$$

[Note]

ΔA_{590}: $\Delta A_{Sample} - \Delta A_{Blank}$ (ΔA_{Blank} is the ΔA when the standard concentration is 0).

f: Dilution factor of sample before test.

C_{pr}: Protein concentration of 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 (μmol H2O2 Equiv./L)	5.60	34.80	85.00
%CV	2.6	2.2	2.1

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 (μmol H2O2 Equiv./L)	5.60	34.80	85.00
%CV	3.2	3.6	3.7

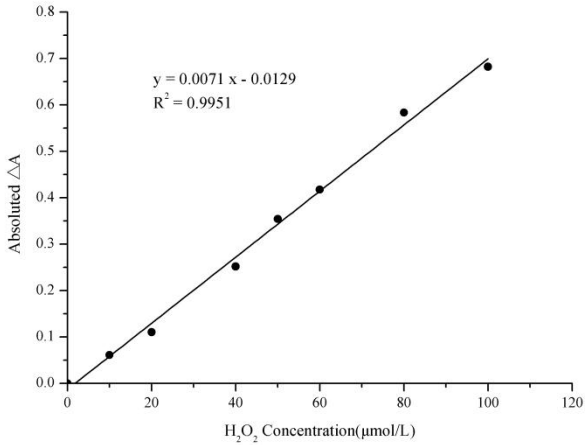
Sensitivity

The analytical sensitivity of the assay is 2.5 μmol H2O2 Equiv./L. 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.

2. Standard curve:

As the ΔA value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
Average ΔA	0.085	0.146	0.195	0.337	0.439	0.502	0.668	0.767
Absoluted ΔA	0.000	0.061	0.110	0.252	0.354	0.417	0.584	0.682



Appendix II Example Analysis

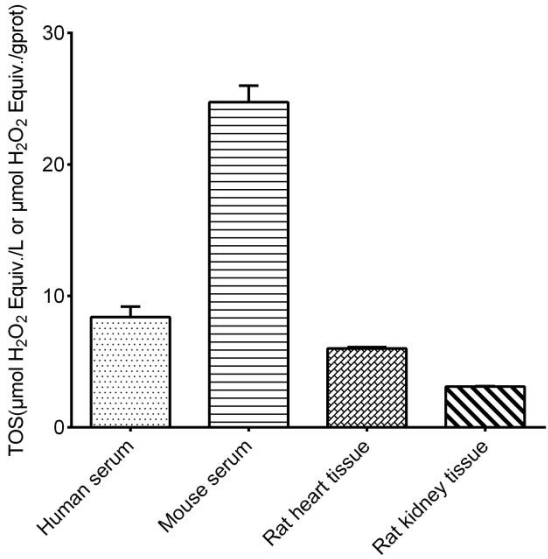
Example analysis：

For human serum, take 20 μL to the sample wells and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.007 x - 0.0146, the ΔA value of the sample is 0.142, the average ΔA value of the blank is 0.098, the absolute ΔA value of the sample: ΔA_{590} = 0.142 - 0.098 = 0.044, and the calculation result is:

TOS (μmol H_2O_2 Equiv./L) =（0.044 + 0.0146）÷ 0.007 = 8.37 μmol H_2O_2 Equiv./L

Detect human serum, mouse serum, 10% rat heart tissue homogenate (the concentration of protein is 8.42 gprot/L), and 10% rat kidney tissue homogenate (the concentration of protein is 11.71 gprot/L) according to the protocol, the result is as follows：



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.

Appendix III Products Cited In Published Literatures

1. Guler A , Yilmaz A , Oncer N ,et al.Machine learning-assisted SERS approach enables the biochemical discrimination in Bcl-2 and Mcl-1 expressing yeast cells treated with ketoconazole and fluconazole antifungals[J].Talanta, 2024, 276.DOI:10.1016/j.talanta.2024.126248.

2. Oi-Jurjevi B , Borkovi-Miti S , Pavlovi S ,et al.Lemon Flavonoid Extract Eriomin Improves Pro/Antioxidant Status and Interferes with Cholesterol Metabolism without Affecting Serum Cholesterol Levels in Aged Rats[J].International Journal of Molecular Sciences, 2024, 25(10).DOI:10.3390/ijms25105221.

3. Prvulovic M , Pavlovic S , Mitic S B ,et al.Mitigating the effects of time in the heart and liver: The variable effects of short- and long-term caloric restriction[J].Mechanisms of Ageing and Development, 2024, 222.DOI:10.1016/j.mad.2024.111992.

4. Bozkurt A S , Tilmaz S G .Ferroptotic Potency of ISM1 Expression in the Drug-Induced Alzheimer's Disease-Like Phenotype Under the Influence of Betulin[J].Journal of Alzheimer's disease: JAD, 2023, 96(4):1565-1578.DOI:10.3233/JAD-230940.

5. Ayenur Güler, Yardmc B K , Zek N I .Human anti-apoptotic Bcl-2 and Bcl-xL proteins protect yeast cells from aging induced oxidative stress [J].Biochimie [2025-02-10]. DOI: 10.1016/j.biochi.2024.10.009.