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

Catalog No：E-BC-K136-S

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

Measuring instrument：Spectrophotometer（520 nm）

Detection range：0.62-145.2 U/mL

Elabscience® Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit

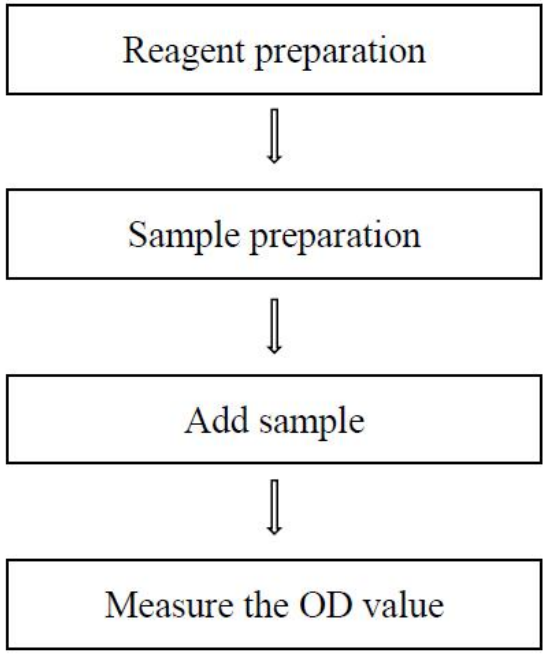
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 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.

Table of contents

Assay summary



Intended use

This kit can be used for detection of total antioxidant capacity (T-AOC) in serum, plasma, saliva, urine, tissue and cells samples.

Detection principle

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflect the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent	Powder ×1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	Ferric Salt Stock Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8°C, 12 months, shading light
Reagent 4	Ferric Salt Diluent	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 5	Stop Solution	24 mL × 1 vial	24 mL ×1 vial	2-8°C, 12 months
Reagent 6	Clarificant	24 mL × 1 vial	24 mL × 1 vial	2-8°C, 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:

Microplate reader (500-520 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic working solution：
Dissolve one vial of chromogenic agent with 120 mL of double distilled water, it can be dissolved by incubating in 80-90°C water bath and used after cooling to room temperature. Store at 2-8°C for 7 days.

③ The preparation of ferric salt working solution：
For each tube, prepare 500 μL of ferric salt working solution (mix well 25 μL of ferric salt stock solution and 475 μL of ferric salt diluent). The ferric salt working solution should be prepared on spot and protect from light. Store at 2-8°C for 2 days.

④ Clarificant will be freeze in cold weather, dissolve by incubating in 37°C water bath until clarification before experiment.

Sample preparation

① Sample preparation

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

Saliva sample: 30 min after gargled, collect the fresh saliva sample, centrifuge at 10000×g for 10 min at 4°C, take the supernatant to preserve it on ice for detection. If not detected on the same day, the saliva can be stored at -80°C for a month.

Urine sample: Collect the fresh urine sample, centrifuge at 10000×g for 10 min at 4°C, take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 40 mg).

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

③ Homogenize 40 mg tissue in 360 μL PBS(0.01 M，pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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 300-500 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min 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
10% Mouse liver tissue homogenization	1
10% Mouse lung tissue homogenization	1
Human saliva	1
Human serum	1
Porcine serum	1
Rat plasma	1
HepG2 cells homogenization	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

The supernatant of sample preparation after centrifugation must be clarified, otherwise centrifuge again.

Operating steps

1. For serum (plasma) and other liquid samples

① Sample tube: Add 1 mL of buffer solution to 5 mL EP tube.
Control tube: Add 1 mL of buffer solution to 5 mL EP tube.

② Sample tube: Add A* mL of sample to the tube.
Control tube: Add nothing.

③ Add 2.0 mL of chromogenic working solution and 0.5 mL of ferric salt working solution to sample tube and control tube.

④ Mix fully and incubate the tubes at 37°C for 30 min.

⑤ Add 0.1 mL of stop solution to sample tube and control tube.

⑥ Sample tube: Add nothing.
Control tube: Add A mL of sample to the tube.

⑦ Mix well and stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz cuvette.

Note: For serum or plasma sample, it is recommended that A* is 0.1 mL.

2. For tissue and cells samples

① Sample tube: Add 1 mL of buffer solution to 5 mL EP tube.
Control tube: Add 1 mL of buffer solution to 5 mL EP tube.

② Sample tube: Add A* mL of sample to the tube.
Control tube: Add nothing.

③ Add 2.0 mL of chromogenic working solution and 0.5 mL of ferric salt working solution to sample tube and control tube.

④ Mix well and incubate the tubes at 37°C for 30 min.

⑤ Add 200 μL of stop solution to sample tube and control tube.

⑥ Sample tube: Add nothing.
Control tube: Add A* mL of sample to the tube.

⑦ Add 200 μL of clarificant to sample tube and control tube.

⑧ Mix fully and stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz cuvette.

Note: It is recommended that A* is 0.1-0.2 mL.

Calculation

The sample:

1. Serum (plasma) and other liquid samples

Definition: At 37 °C, the OD value of the reaction system was increased 0.01 by 1 mL of sample per minute is defined as a unit of total antioxidant capacity.

$$\text{T-AOC activity (U/mL)} = \frac{\Delta A}{0.01} \div 30^{*} \times \frac{V_1}{V_2} \times f$$

2. Tissue and cell samples

Definition: At 37 °C, the OD value of the reaction system was increased 0.01 by 1 mg of protein per minute is defined as a unit of total antioxidant capacity.

$$\text{T-AOC activity (U/mgprot)} = \frac{\Delta A}{0.01} \div 30^{*} \times \frac{V_1}{V_2} \times f \div C_{pr}$$

[Note]

ΔA: $OD_{Sample} - OD_{Control}$

*: The reaction time, 30 min.

V_1: The total volume of reaction, mL.

V_2: The volume of sample added to the reaction, mL.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, mgprot/mL.

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)	12.50	72.40	126.00
%CV	3.0	2.6	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)	12.50	72.40	126.00
%CV	8.1	7.9	8.6

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/mL)	32.8	88.5	108
Observed Conc. (U/mL)	33.8	93.8	114.5
Recovery rate (%)	103	106	106

Sensitivity

The analytical sensitivity of the assay is 0.62 U/mL. 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.

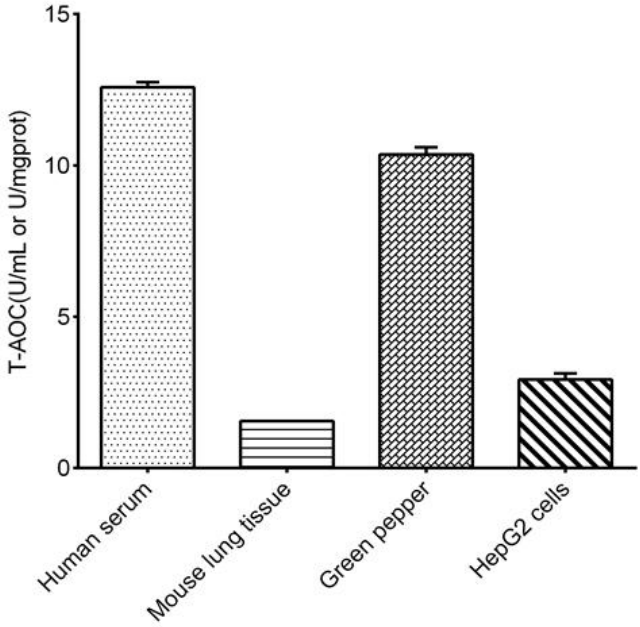
Appendix II Example Analysis

Example analysis：

Take 0.1 mL of human serum and carry the assay according to the operation steps. The results are as follows: the average OD value of the sample is 0.140, the average OD value of the control is 0.038, and the calculation result is:

$$\text{T-AOC activity (U/mL)} = \frac{0.140 - 0.038}{0.01} \div 30 \times \frac{3.7}{0.1} = 12.58(\text{U/mL})$$

Detect human serum (A*=0.1 mL), 10% mouse lung tissue homogenate (the concentration of protein in sample is 5.88 mgprot/mL, A*=0.1 mL), 10% green pepper tissue homogenate (the concentration of protein in sample is 2.73 mgprot/mL, A*=0.1 mL) and HepG2 cells (the concentration of protein in sample is 0.88 mgprot/mL, A*=0.1 mL) according to the protocol, the result is as follows：



Appendix III Publications

1. Bao L , Huang Y , Gu F ,et al.Zearalenone induces liver injury in mice through ferroptosis pathway[J].The Science of the total environment, 952:175875.DOI:10.1016/j.scitotenv. 2024.175875.
2. Nebrisi E E .Phytochemical Evaluation of Lepidium meyenii, Trigonella foenum-graecum, Spirulina platensis, and Tribulus arabica, and Their Potential Effect on Monosodium Glutamate Induced Male Reproductive Dysfunction in Adult Wistar Rats[J].Antioxidants, 2024, 13.DOI:10.3390/antiox13080939.
3. Cao Y , Yang Y , Liang Z ,et al.Synthesis of Ganoderic Acids Loaded Zein-Chitosan Nanoparticles and Evaluation of Their Hepatoprotective Effect on Mice Given Excessive Alcohol[J]. 2024.
4. Govender S , Kruger M J , Vyver M V D .Counteracting diabetes-induced adipose tissue derived-stromal cell senescence[J].Biochimie, 2024, 220(000):11.DOI:10.1016/j.biochi. 2023.12.001.
5. Sukkasam N , Kaewbai-Ngam J , Leksingto J ,et al.Disrupted H2synthesis combined with methyl viologen treatment inhibits photosynthetic electron flow to synergistically enhance glycogen accumulation in the cyanobacteriumSynechocystissp. PCC 6803[J].Plant Molecular Biology, 2024, 114(4).DOI:10.1007/s11103-024-01484-3.

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.