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

Catalog No：E-BC-K219-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（405-425 nm）

Detection range：0.047-1.50 mmol/L

Elabscience® Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit (ABTS, Enzyme Method)

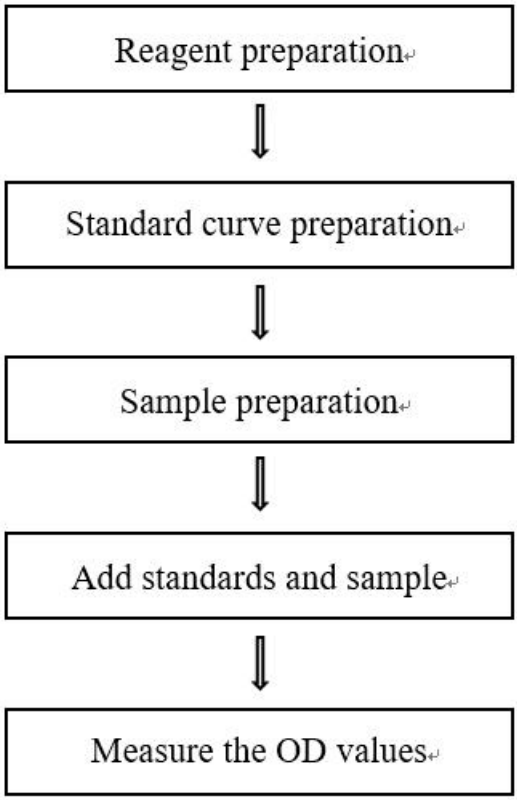
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure total antioxidant capacity (T-AOC) in serum, plasma, tissue, cells or other sample.

Detection principle

The principle of the ABTS method for determining the T-AOC is as follows. ABTS is oxidized to green $ABTS^+$ by appropriate oxidant, which can be inhibited if there exist antioxidants. The T-AOC of the sample can be determined and calculated by measuring the absorbance of $ABTS^+$ at 414 nm or 734 nm. Trolox is an analog of VE and has a similar antioxidant capacity to that of VE. Trolox is used as a reference for other antioxidant antioxidants. For example, the T-AOC of Trolox is 1, then the antioxidant capacity of the other substance with the same concentration is showed by the ratio of its antioxidant capacity to Trolox antioxidant capacity.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	24 mL × 1 vial	2-8℃, 12 months
Reagent 2	ABTS Solution	0.5 mL × 1 vial	1 mL × 1 vial	2-8℃, 12 months shading light
Reagent 3	H_2O_2 Solution	0.25 mL × 1 vial	0.5 mL × 1 vial	2-8℃, 12 months
Reagent 4	Peroxidase	0.1 mL × 1 vial	0.2 mL × 1 vial	2-8℃, 12 months
Reagent 5	5 mmol/L Trolox Standard	0.3 mL × 1 vial	0.6 mL × 1 vial	-20℃, 12 months shading light
	Microplate	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(405-425 nm), Micropipettor

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), 60% Ethanol

Reagent preparation

① The preparation of H_2O_2 application solution:
Before testing, please prepare sufficient H_2O_2 application solution according to the test wells. For example, prepare 200 μL of H_2O_2 application solution (mix well 5 μL of H_2O_2 solution and 195 μL of double distilled water). The H_2O_2 application solution should be prepared on spot.

② The preparation of ABTS working solution:
For each well, prepare 170 μL of ABTS working solution (mix well 152 μL of buffer solution, 10 μL of ABTS solution and 8 μL of H_2O_2 application solution). Store the prepared solution at room temperature with shading light and run out in 30 min.

③ The preparation of peroxidase application solution:
For each well, prepare 20 μL of peroxidase application solution (mix well 2 μL of peroxidase and 18 μL of buffer solution). Store the prepared solution at room temperature with shading light and run out in 30 min.

④ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 5 mmol/L Trolox Standard with PBS or 60% ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mmol/L. (If the sample to be tested is water-soluble, dilute the standard with PBS. If the sample to be tested is water-insoluble, dilute the standard with 60% ethanol.) Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**
5 mmol/L Trolox Standard (μL)	0	4	8	12	16	24	32	40
Diluent (μL)	200	196	192	188	184	176	168	160

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 10 min at 4℃. Take the supernatant to preserve 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 60% ethanol 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 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℃.

④ Centrifuge at 10000×g for 10 minutes 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% Rat brain tissue homogenization	1
10% Rat liver tissue homogenization	1
10% Rat kidney tissue homogenization	1
10% Epipremnum aureum tissue homogenization	1
Human serum	1
Human saliva	1
Human urine	1
Rat serum	1

Note: When the sample was water soluble, the diluent is PBS (0.01 M, pH 7.4); When the sample is insoluble, the diluent is 60% ethanol. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

ABTS working solution should be stored at room temperature with shading light and run out in 30 min.

Operating steps

① Standard well: Add 10 μL of standard with different concentration into the standard wells.
Sample well: Add 10 μL of sample into the sample wells.

② Add 20 μL of peroxidase application solution to each well.

③ Add 170 μL of ABTS working solution to each well.

④ Mix well and stand for 6 min at room temperature. Measure the OD values of each well at 414 nm with Microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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. Serum (plasma) and other liquid sample:

$$\text{T-AOC}\ (\text{mmol/L}) = (A_{414} - b) \div a \times f$$

2. Tissue sample:

$$\text{T-AOC}\ (\text{mmol/kg wet weight}) = (A_{414} - b) \div a \div \frac{m}{V} \times f$$

3. Cells sample:

$$\text{T-AOC}\ (\text{mmol/gprot}) = (A_{414} - b) \div a \div C_{pr} \times f$$

[Note]

A_{414}: Average OD of sample.

f: Dilution factor of sample before test.

V: The volume of sample homogenate, mL.

m: The weight of tissue sample, g.

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 (mmol/L)	0.12	0.58	1.20
%CV	2.5	2.3	1.8

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 (mmol/L)	0.12	0.58	1.20
%CV	4.0	3.9	4.4

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 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.33	0.75
Observed Conc. (mmol/L)	0.2	0.3	0.8
Recovery rate (%)	101	105	100

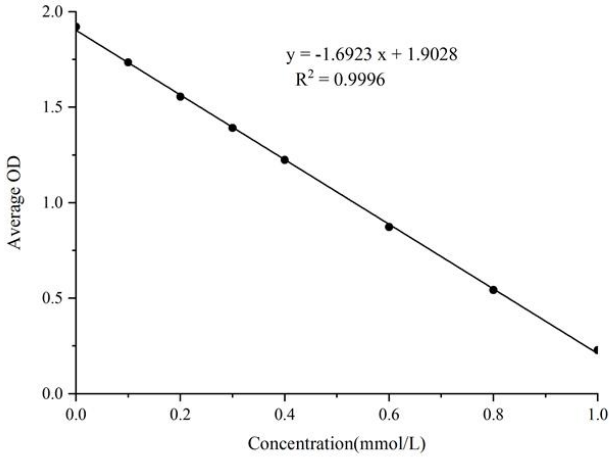
Sensitivity

The analytical sensitivity of the assay is 0.047 mmol/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 OD 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 (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**
Average OD	1.921	1.735	1.555	1.392	1.224	0.873	0.543



Appendix II Example Analysis

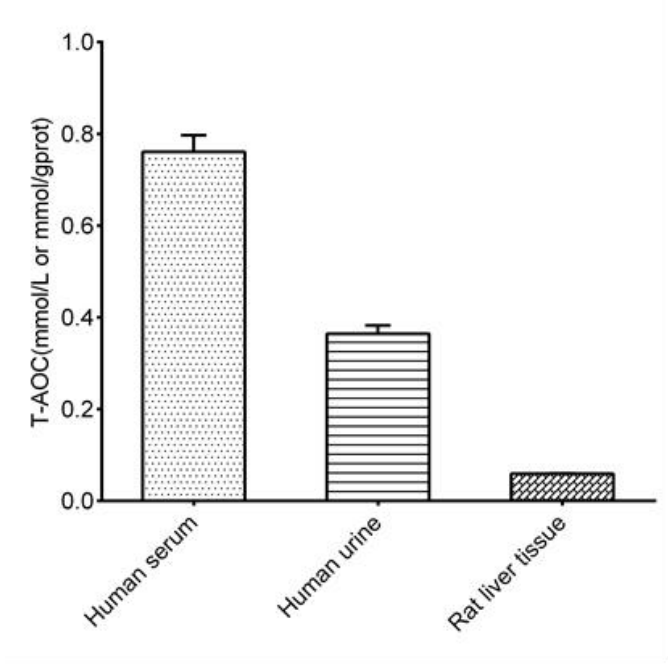
Example analysis:

Take 10 μL of human serum, carry the assay according to the operation steps. The results are as follows:

Standard curve: y = -1.122 x + 1.7172, the average OD value of the sample is 0.863, and the calculation result is:

$$\text{T-AOC (mmol/L)} = (0.863 - 1.7172) \div (-1.122) = 0.76 \text{ mmol/L}$$

Detect human serum, 10% rat liver tissue homogenate (the concentration of protein is 15.14 gprot/L), human urine 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.