Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit

Catalog No: E-BC-K136-M

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.62 U/mL

Detection range: 0.62-190.43 U/mL

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

The kit is used for the determination of total antioxidant capacity (T-AOC) in serum, plasma, whole blood, tissue, cell and culture supernatant samples.

▲Background

There are two kinds of antioxidant system, one is enzyme antioxidant system, including superoxide dismutase (SOD),) catalase (CAT), glutathione peroxidase (GSH-Px). The other is non-enzymatic antioxidant systems, including uric acid, vitamin C, vitamin E, glutathione, bilirubin, α-lipoic acid, carotenoid. Antioxidant capacity is thought to be the cumulative effect of all antioxidants in blood and body fluids.

▲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	Specification	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Chromogenic Agent	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 3	Ferric Salt Stock Solution	0.4 mL × 1 vial	2-8℃ , 6 months, shading light
Reagent 4	Ferric Salt Diluent	8 mL × 1 vial	2-8℃ , 6 months
Reagent 5	Stop Solution	1.25 mL × 2 vials	2-8℃ , 6 months
Reagent 6	Clarificant	1.25 mL × 2 vials	2-8℃ , 6 months
	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.
If you have any problem, please contact our Technical Service Center for help.
Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents:

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key point of the assay

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

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 working solution:
   Dissolve a vial of powder with 20 mL of double distilled water fully (It can be dissolved by incubating in 80-90℃ water bath). It can be used after cooling to room temperature.
2. Preparation of reagent 3 working solution:
   Dilute the reagent 3 with reagent 4 at the ratio of 1:19. Prepared the fresh solution before use.
3. Reagent 6 will be freeze in cold weather, dissolve by incubating in 37℃ water bath until clarification before experiment.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The sample should not contain DTT, 2-mercaptoethanol and other reducing agents.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.62-190.43 U/mL).

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

Sample type	Dilution factor
Human serum	1
Human urine	1-2
10% Rat liver tissue homogenate	1
10% Epipremnum aureum tissue homogenate	1
HepG2 cells	1
HepG2 cell culture supernatant	1

Note:The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	520 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don ’ t add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

[Note]： S1-S48, sample wells; S1'-S48', control wells.

▲Operating steps

For serum (plasma) and other liquid samples

(1) Sample tube: Add 100 μL of reagent 1 to 1.5 mL EP tube.
Control tube: Add 100 μL of reagent 1 to 1.5 mL EP tube.

(2) Sample tube: Add 10 μL of sample to the tube.
Control tube: Add nothing.

(3) Add 200 μL of reagent 2 working solution and 50 μL of reagent 3 working solution to sample tube and control tube.

(4) Mix fully and incubate the tubes at 37℃ for 30 min.

(5) Add 10 μL of reagent 5 to sample tube and control tube.

(6) Sample tube: Add nothing.
Control tube: Add 10 μL of sample to the tube.

(7) Mix fully and stand for 10 min at room temperature. Take 300 μL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.

For tissue and cells samples

(1) Sample tube: Add 100 μL of reagent 1 to 1.5 mL EP tube.
Control tube: Add 100 μL of reagent 1 to 1.5 mL EP tube.

(2) Sample tube: Add 10 μL of sample to the tube.
Control tube: Add nothing.

(3) Add 200 μL of reagent 2 working solution and 50 μL of reagent 3 working solution to sample tube and control tube.

(4) Mix fully and incubate the tubes at 37℃ for 30 min.

(5) Add 20 μL of reagent 5 to sample tube and control tube.

(6) Sample tube: Add nothing.
Control tube: Add 10 μL of sample to the tube.

(7) Add 20 μL of reagent 6 to sample tube and control tube.

(8) Mix fully and stand for 10 min at room temperature. Take 300 μL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.

For whole blood samples

(1) Sample tube: Add 100 μL of reagent 1 to 1.5 mL EP tube.
Control tube: Add 100 μL of reagent 1 to 1.5 mL EP tube.

(2) Sample tube: Add 10 μL of sample to the tube.
Control tube: Add nothing.

(3) Add 200 μL of reagent 2 working solution and 50 μL of reagent 3 working solution to sample tube and control tube.

(4) Mix fully and incubate the tubes at 37℃ for 30 min.

(5) Add 20 μL of reagent 5 to sample tube and control tube.

(6) Sample tube: Add nothing.
Control tube: Add 10 μL of sample to the tube.

(7) Mix fully and stand for 10 min at room temperature. Take 300 μL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.

▲Operation table

For serum (plasma) and other liquid samples

	Sample tube	Control tube
Reagent 1 (μL)	100	100
Sample (μL)	10	
Reagent 2 working solution (μL)	200	200
Reagent 3 working solution (μL)	50	50
Mix fully, react at 37℃ for 30 min.		
Reagent 5 (μL)	10	10
Sample (μL)		10
Mix fully and stand for 10 min at room temperature. Take 300 μL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.		

For tissue and cells samples

	Sample tube	Control tube
Reagent 1 (μL)	100	100
Sample (μL)	10	
Reagent 2 working solution (μL)	200	200
Reagent 3 working solution (μL)	50	50
Mix fully, react at 37℃ for 30 min.		
Reagent 5 (μL)	20	20
Sample (μL)		10
Reagent 6 (μL)	20	20
Mix fully and stand for 10 min at room temperature. Take 300 μL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.		

For whole blood samples

	Sample tube	Control tube
Reagent 1 (μL)	100	100
Sample (μL)	10	
Reagent 2 working solution (μL)	200	200
Reagent 3 working solution (μL)	50	50
Mix fully, react at 37℃ for 30 min.		
Reagent 5 (μL)	20	20
Sample (μL)		10
Mix fully and stand for 10 min at room temperature. Take 300 μL of reaction liquid to 96 well microplate and measure the OD value of each well at 520 nm with microplate reader.		

▲Calculation

1.Serum (plasma), whole blood and other liquid samples

Definition: At 37 ℃ , 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.

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

2.Tissue and cell samples

Definition: At 37℃ , 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.

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

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.62-190.43 U/mL	Average intra-assay CV (%)	4.8
Sensitivity	0.62 U/mL	Average inter-assay CV (%)	5.6
Average recovery rate (%)	96		

▲Example analysis

Take 10 μL of human serum sample and carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.081, the average OD value of the control is 0.020, and the calculation result is:

$$\text{T-AOC (U/mL)} = \frac{0.081-0.020}{0.01} \div 30 \times \frac{0.37}{0.01} = 7.52\ \text{U/mL}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 1000-2000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Whole blood

Collect the fresh blood to the test tube containing anticoagulant ($V_{anticoagulant}$:V_{blood}=1:9), mix gently. And the sample can be stored at 2-8℃ for 1-2 days.

▲Cell culture supernatant

Detect the cell culture supernatant directly. If there is turbidity, centrifuge at 3100 g for 10 min, take the supernatant and preserve it on ice for detection. If not detected on the same day, stored the serum at -80℃ , which can be stored for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^5): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 10 min).

▲ Notes for sample

1. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
2. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.