Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit

Catalog No: E-BC-K136-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 50 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.62 U/mL

Detection range: 0.62-145.2 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

This kit can be used for detection of total antioxidant capacity (T-AOC) in serum, plasma, saliva, urine, tissue and cells 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	60 mL× 2 vials	2-8℃ , 6 months
Reagent 2	Chromogenic Agent	Powder × 2 vials	2-8°C , 6 months, shading light
Reagent 3	Ferric Salt Stock Solution	1.5 mL × 2 vials	2-8°C , 6 months, shading light
Reagent 4	Ferric Salt Diluent	60 mL × 1 vial	2-8°C , 6 months
Reagent 5	Stop Solution	24 mL × 1 vial	2-8°C , 6 months
Reagent 6	Clarificant	24 mL × 1 vial	2-8°C , 6 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.
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

Spectrophotometer (520 nm), Vortex mixer, Micropipettor, Water bath, Incubator, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 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 reagent 2 with 120 mL double distilled water fully (It can be dissolved by incubating in 80-90°C 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 working solution before use.
3. Reagent 6 will be freeze in cold weather, dissolve by incubating in 37°C water bath till 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 (0.62-145.2 U/mL).

Assay protocol	
Ambient temperature	25-30°C
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

▲For serum, plasma or other liquid sample

Operating steps

(1) Sample tube: Add 1.0 mL of reagent 1 to 5 mL EP tube.
Control tube: Add 1.0 mL of reagent 1 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 reagent 2 working solution and 0.5 mL of reagent 3 working solution to each tube.

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

(5) Add 0.1 mL of reagent 5 to each tube.

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

(7) 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.

Operation table

	Sample tube	Control tube
Reagent 1 (mL)	1.0	1.0
Sample (mL)	A*	
Reagent 2 working solution (mL)	2.0	2.0
Reagent 3 working solution (mL)	0.5	0.5
Mix fully and incubate the tubes at 37°C for 30 min.		
Reagent 5 (mL)	0.1	0.1
Sample (mL)		A*
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: For serum or plasma sample, it is recommended that A* is 0.1 mL.

▲For tissue and cell sample

Operating steps

(1) Sample tube: Add 1.0 mL of reagent 1 to 5 mL EP tube.
Control tube: Add 1.0 mL of reagent 1 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 reagent 2 working solution and 0.5 mL of reagent 3 working solution to each tube.

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

(5) Add 0.2 mL of reagent 5 to sample tube and control tube.

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

(7) Add 0.2 mL of reagent 6 to each 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.

Operation table

	Sample tube	Control tube
Reagent 1 (mL)	1.0	1.0
Sample (mL)	A*	
Reagent 2 working solution (mL)	2.0	2.0
Reagent 3 working solution (mL)	0.5	0.5
Mix fully and incubate the tubes at 37°C for 30 min.		
Reagent 5 (mL)	0.2	0.2
Sample (mL)		A*
Reagent 6 (mL)	0.2	0.2
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

1. For serum, plasma or other liquid sample

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.

$$\underset{(U/mL)}{T-AOC\ activity}=\frac{\Delta A}{0.01}\div 30'\times\frac{V_1}{V_2}\times f$$

2. For tissue and cell samples

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

$$\underset{(U/mgprot)}{T-AOC\ activity}=\frac{\Delta A}{0.01}\div 30'\times\frac{V_1}{V_2}\times f\div C_{pr}$$

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	0.62-145.2 U/mL	Average intra-assay CV (%)	2.7
Sensitivity	0.62 U/mL	Average inter-assay CV (%)	8.2
Average recovery rate (%)	105		

▲Inter-assay CV

Take three kits of different batches to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 3 times (n=3) parallelly with each kit. Then calculate the corresponding values according to the following formula. The average Inter-assay CV is 8.2%.

$$\overline{x}_T = \frac{\overline{x}_1 + \overline{x}_2 + \overline{x}_3}{3}$$

$$R = \frac{\overline{x}_{max} - \overline{x}_{min}}{\overline{x}_T} \times 100\%$$

$$\overline{R} = \frac{R_1 + R_2 + R_3}{3} \quad (n=3)$$

$\overline{x}_{max}$--- The max values of $\overline{x}_i$

$\overline{x}_{min}$--- The min values of $\overline{x}_i$

$\overline{x}_T$---The average values of $\overline{x}_i$

R_i---The value of each batch number kit

▲Intra-assay CV

Take one kit to detect the samples of high concentration, middle concentration and low concentration respectively. The concentrations should be within the detection range. The samples of each concentration were tested 6 times (n=6) parallelly, and the average Intra-assay CV is 2.7%, which was calculated according to the following formula.

$$CV = \frac{S}{\bar{x}} \times 100\%$$

S--- Standard deviation

▲Sensitivity

The volume of sample added to reaction is 0.1 mL, carry the assay according to the operation table, the minimum detected absorbance is 0.005 (ΔA_{520}=0.005), calculate the sensitivity according to the formula in calculation is 0.62 U/mL.

▲Recovery rate

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%.

▲Example analysis

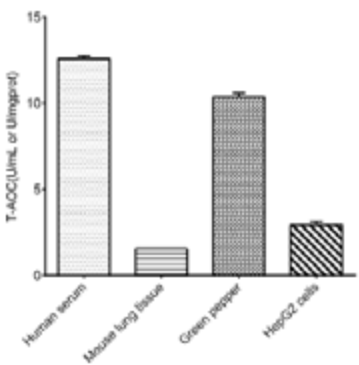
Take 0.1 mL of human serum and carry the assay according to the operation table.

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 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, 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.

▲ Saliva sample

30 min after gargled, collect the fresh saliva sample, 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 plasma can be stored at -80℃ for a month.

▲ Urine sample

Collect the fresh urine sample, 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 plasma can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C 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 (10^6): 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, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or normal saline.
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 cm^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).

▲ Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. 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.

Appendix III References

1. Sies H. Physiological Society Symposium: Impaired Endothlial and Smooth Muscle Cell Function in Oxidative Stress - Oxidative Stress: Oxidants and Antioxidants. Experitnental Physiology, 1997, 82: 291-295.
2. Bartosz G. Total antioxidant capacity. Advances in Clinical Chemistry, 2003, 37(37): 219-272.
3. Smith R, Vantman D, Ponce J, et al. Andrology: Total antioxidant capacity of human seminal plasma. Human Reproduction, 1996, 11(8): 1655-1660.