Technical Manual

Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit

- Catalogue Code: MAES0109
- Size: 96T
- Research Use Only

1.Key features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.62-190.43 U/mL

Sensitivity:

0.62 U/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. 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.

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	12 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	Lyophilized × 2 vials	2-8°C, 6 months, Avoid direct sunlight
Ferric Salt Stock Solution	0.4 mL × 1 vial	2-8°C, 6 months, Avoid direct sunlight
Ferric Salt Diluent	8 mL × 1 vial	2-8°C, 6 months
Stop Solution	1.25 mL × 2 vials	2-8°C, 6 months
Clarificant	1.25 mL × 2 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (495-525 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

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

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **chromogenic agent working solution:** Dissolve a vial of chromogenic agent powder with 20 mL of 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.

3. Preparation of ferric salt working solution: Dilute the ferric salt stock solution with ferric salt diluent at the ratio of 1:19. Prepared the fresh solution before use.

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

8. Sample Preparation

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

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C Take the serum (which is the upper light yellow clarified liquid layer and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Cell culture supernatant:
Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant and preserve on ice before detection. If not detected on the same day, it can be stored at -80°C for a month.

4. 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°C for 1-2 days.

5. Cell sample:
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 (2×10^6): Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (μL) =1: 300. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

6. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (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 and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:
The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:
Large variances in results may be seen when performing pre-experiments. 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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 520 nm

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.

10. Operation Steps

For serum (plasma) and other liquid samples

1. **Sample tube:** Add 100 µL of buffer solution to 1.5 mL EP tube.
   Control tube: Add 100 µL of buffer solution 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 chromogenic agent working solution and 50 µL 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 10 µL of stop solution 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

	Sample tube	Control tube
Buffer solution (µL)	100	100
Sample (µL)	10	
Chromogenic agent working solution (µL)	200	200
Ferric salt working solution (µL)	50	50
Mix fully, react at 37°C for 30 min.		
Stop solution (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

1. **Sample tube:** Add 100 µL of buffer solution to 1.5 mL EP tube.
   Control tube: Add 100 µL of buffer solution 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 chromogenic agent working solution and 50 µL 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 20 µL of stop solution 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 clarificant 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.

Operation Table

	Sample tube	Control tube
Buffer solution (µL)	100	100
Sample (µL)	10	
Chromogenic agent working solution (µL)	200	200
Ferric salt working solution (µL)	50	50
Mix fully, react at 37°C for 30 min.		
Stop solution (µL)	20	20
Sample (µL)		10
Clarificant (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

1. **Sample tube:** Add 100 µL of buffer solution to 1.5 mL EP tube.
   Control tube: Add 100 µL of buffer solution 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 chromogenic agent working solution and 50 µL 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 20 µL of stop solution to sample tube and control tube.

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

Operation Table

	Sample tube	Control tube
Buffer solution (µL)	100	100
Sample (µL)	10	
Chromogenic agent working solution (µL)	200	200
Ferric salt working solution (µL)	50	50
Mix fully, react at 37°C for 30 min.		
Stop solution (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.		

11. Calculations

1. Serum (plasma), whole blood 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.

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

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

2. Tissue and cell sample:

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.

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

12. Performance Characteristics

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

Analysis

Take 10 μL of human serum, 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:

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

$$= 7.52 \text{ U/mL}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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