Technical Manual

Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit (ABTS, Chemical Method)

- Catalogue Code: MAES0168
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.05-1.00 mmol/L

Sensitivity:

0.05 mmol/L

Storage:

-20°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, urine, saliva, tissue, cells and other sample.

4. 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 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.

5. Kit components & storage

Item	Specification	Storage
ABTS Solution	0.6 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Oxidant Solution	0.6 mL × 1 vial	-20°C, 6 months
Trolox Standard (5 mmol/L)	0.5 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
10×PBS Solution	1.5 mL × 2 vials	-20°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (730-740 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- 80% Ethanol

6. Assay Notes:

1. ABTS solution and ABTS working solution should be stored away from direct sunlight, otherwise the OD value will be decreased.

2. If the sample to be tested is water-soluble, dilute the standard and samples with PBS. If the sample to be tested is water-insoluble, dilute the standard and samples with 80% ethanol.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **concentrated ABTS working solution:** Mix the ABTS solution and oxidant solution at a ratio of 1: 1 fully, the concentrated ABTS working solution can be use after store at room temperature with avoid direct sunlight for 12-16 hours. The prepared solution can be stored at 2-8°C for 2 days.

3. Preparation of **1×PBS solution:** Dilute the 10×PBS solution with double distilled water for 10 times.

4. Preparation of **ABTS working solution:** Dilute the concentrated ABTS working solution with 1×PBS or 80% ethanol (self-prepared), the absorbance at 734 nm of blank tube (10 μL diluent+200 μL ABTS working solution) should be 0.7±0.05.
   Note: If the sample to be tested is water-soluble, the diluent is PBS, dilute concentrated ABTS working solution with PBS for 20-30 times. If the sample to be tested is water-insoluble, the diluent is 80% ethanol (self-prepared), dilute concentrated ABTS working solution with 80% ethanol for 25-35 times.

8. Sample Preparation

Sample requirements: SDS, Tween, Triton, NP-40 and other detergents should not be added to the samples, and DTT, 2-mercaptoethanol and other reducing substances should not be added.

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. 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°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 1000-2000 g 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) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 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.

4. 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): PBS (0.01 M, pH 7.4) (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

5. 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 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 to preserve it on ice for 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 (0.05-1.00 mmol/L).

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

Sample Type:	Dilution Factor
20% Tomato tissue homogenization	2-5
10% Mouse heart tissue homogenization	8-12
10% Mouse liver tissue homogenization	8-12
10% Mouse lung tissue homogenization	8-12
Human saliva	2-5
Human urine	15-30
Human serum	15-30
Human plasma	8-15

Note: The diluent is 1 × PBS or 80% ethanol.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 734 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute trolox standard (5 mmol/L) with 1×PBS or 80% ethanol (self-prepared) to a serial concentration. The recommended dilution gradient is as follows: 0, 0.15, 0.3, 0.45, 0.6, 0.75, 0.9, 1 mmol/L. (If the sample to be tested is water-soluble, dilute the standard and samples with PBS. If the sample to be tested is water-insoluble, dilute the standard and samples with 80% ethanol.)

The measurement of samples

1. **Standard tube:** Add 10 μL of standard with different concentration to the corresponding well.
   Sample tube: Add 10 μL of sample to the corresponding well.

2. Add 200 μL of ABTS working solution to each well.

3. Mix fully and stand for 2-6 min at room temperature. Measure the OD values of each well at 734 nm with microplate reader.

Operation Table

	Standard well	Sample well
Standards with different concentrations (μL)	10	
Sample (μL)		10
ABTS working solution (μL)	200	200
Mix fully and stand for 2-6 min at room temperature. Measure the OD values of each well at 734 nm with microplate reader.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

$$\text{T-AOC}_{(\text{mmol/L})} = (A_{734}\text{-b}) \div a \times f$$

2. Tissue and cell sample:

$$\text{T-AOC}_{(\text{mmol/gprot})} = (A_{734}\text{-b}) \div a \div C_{pr} \times f$$

y: $OD_{Blank} - OD_{Standard}$

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

A_{734}: $OD_{Blank} - OD_{Sample}$

f: Dilution factor of sample before test

Cpr: Concentration of protein in sample, gprot/L

12. Performance Characteristics

Detection Range	0.05-1.00 mmol/L
Sensitivity	0.05 mmol/L
Average recovery rate (%)	102
Average inter-assay CV (%)	5.0
Average intra-assay CV (%)	4.1

Analysis

Dilute the human plasma with 1×PBS for 12 times, then take 10 μL of diluted sample, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.67037 x – 0.00485, the average OD value of the sample well is 0.2510, the average OD value of the blank well is 0.6964, and the calculation result is:

T-AOC (mmol/L) = (0.6964-0.2510+0.00485) ÷ 0.6703×12=8.06 (mmol/L)

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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