Technical Manual

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

- Catalogue Code: MAES0147
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.047-1.50 mmol/L

Sensitivity:

0.047 mmol/L

Storage:

2-8°C and -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.

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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, tissue, cells or 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 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.

5. Kit Components & Storage:

Item	Specification	Storage
Buffer Solution	24 mL × 1 vial	2-8°C, 6 months
ABTS Solution	1 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
H_2O_2 Solution	0.5 mL × 1 vial	2-8°C, 6 months
Peroxidase	0.2 mL × 1 vial	2-8°C, 6 months
Trolox Standard (5 mmol/L)	0.6 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Microplate Reader (405-425 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)
- 80% Ethanol

6. Assay Notes:

ABTS working solution should be stored at room temperature, avoiding direct sunlight and run out in 30 min.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **H_2O_2 application solution**: Dilute H_2O_2 Solution with double distilled water at a ratio of 1:39. Prepare fresh solution before use.

3. Preparation of **ABTS working solution**: Prepare the needed amount of ABTS working solution according to the ratio (buffer solution: ABTS solution: H_2O_2 application solution = 152:10:8). Store the prepared solution at room temperature, avoiding direct sunlight and run out in 30 min.

4. Preparation of **Peroxidase application solution**: Dilute peroxidase with buffer solution at ratio of 1:9 before use. Prepare fresh solution before use.

8. Sample Preparation:

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 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 (10^6): PBS (0.01 M, pH 7.4) or 0.9% NaCl (μL) =1: 400. 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.

4. 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 PBS (0.01 M, pH 7.4) or 0.9% NaCl (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 (0.047-1.50 mmol/L).

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 80% ethanol.

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9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 414 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, blank well; B-H, standard wells; S1-S80, sample wells.

10. Operation Steps:

The preparation of standard curve

Dilute 5 mmol/L trolox standard with PBS or 80% ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1, 1.25, 1.5 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 80% ethanol.)

The measurement of samples

1. **Standard well:** Add 10 μL of standard with different concentration to the 96 well micro-plate.
   Sample well: Add 10 μL of sample to the 96 well micro-plate.

2. Add 20 μL of peroxidase application solution to each well of step 1.

3. Add 170 μL of ABTS working solution to each well of step 2.

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

5. Note: The reagent should be added to the bottom of microplate slowly to prevent gas bubble.

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Operation Table

	Standard well	Sample well
Trolox standard with different concentrations (μL)	10	
Sample (μL)		10
Peroxidase application solution (μL)	20	20
ABTS working solution (mL)	170	170
Mix fully and stand for 6 min at room temperature. Measure the OD values of each well at 414 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. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

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

2. Tissue and cells sample:

$$\text{TG content (mmol/gprot)} = \frac{\Delta A_{414} - b}{a} \times f \div C_{pr}$$

y: The average OD value of standard.
x: The concentration of Standard.
a: The slope of standard curve.
b: The intercept of standard curve.
A_{414}: Average OD of sample.
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample (gprot/L)

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12. Performance Characteristics:

Detection Range	0.047-1.50 mmol/L
Sensitivity	0.047 mmol/L
Average recovery rate (%)	101
Average inter-assay CV (%)	4.1
Average intra-assay CV (%)	2.2

Analysis

Take 10 μL of human serum, carry the assay according to the operation table.

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:

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

$$= 0.76 \text{ mmol/L}$$

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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.

Notes:

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