PRODUCT INFORMATION & MANUAL

Total Antioxidant Capacity/ T-AOC Assay Kit (Colorimetric)

NBP3-25816

For research use only.
Not for diagnostic or therapeutic procedures.

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

Catalog No: NBP3-25816

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.047 mmol/L

Detection range: 0.047-1.50 mmol/L

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 4.1

Average recovery rate (%): 102

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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, tissue, cells or other sample.

▲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

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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	24 mL × 1 vial	2-8℃ , 12 months
Reagent 2	ABTS Solution	1 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 3	H_2O_2 Solution	0.5 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Peroxidase	0.2 mL × 1 vial	2-8℃ , 12 months
Reagent 5	5 mmol/L Trolox Standard	0.6 mL × 1 vial	-20℃ , 12 months, shading light
	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.			

▲Materials prepared by users

Instruments

Microplate reader(405-425 nm), Micropipettor.

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), 60% Ethanol.

⚠ 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

ABTS working solution should be stored at room temperature with shading light and run out in 30 min.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 3 application solution:
   Dilute reagent 3 with double distilled water at a ratio of 1:39. Prepare the fresh solution before use.
2. Preparation of ABTS working solution:
   Prepare the needed amount of ABTS working solution according to the ratio (reagent 1: reagent 2: reagent 3 application solution = 152:10:8). Store the prepared solution at room temperature with shading light and run out in 30 min.
3. Preparation of reagent 4 application solution:
   Dilute reagent 4 with reagent 1 at ratio of 1:9 before use. Prepare the fresh solution before use.

▲Sample preparation

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

Sample requirements

1. Samples should not contain decontamination agents such as SDS, Tween-20, NP-40, Triton, nor reductive reagents such as DTT, 2- mercaptoethanol.

2. Plasma sample can't be anticoagulated with EDTA and sodium citrate. Heparin is recommended as the anticoagulant.

▲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 (0.047-1.50 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 60% ethanol.

Assay protocol

▲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.

▲Detailed operating steps

The preparation of standard curve

Dilute 5 mmol/L Trolox Standard with PBS or 60% ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 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 60% ethanol.) Reference is as follows:

Number	Standard concentrations (mmol/L)	5 mmol/L Trolox (μL)	Diluent (μL)
A	0	0	200
B	0.1	4	196
C	0.2	8	192
D	0.3	12	188
E	0.4	16	184
F	0.6	24	176
G	0.8	32	168
H	1.0	40	160

The measurement of samples

1. Standard well: Add 10 μL of standard with different concentration to the wells.
   Sample well: Add 10 μL of sample to the wells.
2. Add 20 μL of reagent 4 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.

▲Summary operation table

	Standard well	Sample well
Trolox standard with different concentrations (μL)	10	
Sample (μL)		10
Reagent 4 application solution (μL)	20	20
ABTS working solution (μL)	170	170
Mix fully and stand for 6 min at room temperature. Measure the OD values at 414 nm.		

▲Calculation

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:

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

2. Tissue sample:

$$T\text{-}AOC(mmol/kg\ wet\ weight) = (A_{414} - b) \div a \div (m / V) \times f$$

3. Cells sample:

$$T\text{-}AOC(mmol/gprot) = (A_{414} - b) \div a \div C_{pr} \times f$$

Note:

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

V: The volume of sample homogenate, mL

m: The weight of tissue sample, g

C_{pr}: Concentration of protein in sample (gprot/L)

Appendix I Data

▲Example 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:

T-AOC(mmol/L)=(0.863-1.7172)÷(-1.122)=0.76 mmol/L

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.

▲Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01M, pH 7.4) at 2-8℃ to remove blood cells. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of 60% ethanol (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. 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 (0.01M, 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 (0.01M, pH 7.4)(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:

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).

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.