Total Antioxidant Status (TAS)

Colorimetric Assay Kit

Catalog No: E-BC-K801-M

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.23 mmol Trolox Equiv. /L

Detection range: 0.23-2 mmol Trolox Equiv. /L

- 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 status (TAS) in serum, plasma, urine, cellular supernatant, animal and plant tissue samples.

▲Background

Reactive oxygen species (ROS), aerobic organisms, are active products continuously produced in the process of their own metabolism due to the stimulation of internal and external environment. In general, the body has a set of antioxidant defense system to maintain the REDOX balance. The strength of antioxidant status of the body defense system is closely related to the health degree, and its scavenging mechanism can be roughly divided into enzymatic antioxidants and non-enzymatic antioxidants. The total antioxidant status of a system is reflected by the total level of various antioxidant macromolecules, antioxidant small molecules and enzymes.

▲Detection principle

ABTS is oxidized to green $ABTS^{\cdot+}$ by appropriate oxidant, which can be reduced to colorless ABTS in the presence of antioxidants. The TAS of the sample can be determined and calculated by measuring the absorbance of $ABTS^{\cdot+}$ at 660 nm. Trolox is an analog of VE and has a similar antioxidant state to that of VE. Trolox is used as a reference substance for total antioxidant status.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	-20℃ , 6 months
Reagent 2	Chromogenic Agent	5 mL × 1 vial	-20℃ , shading light, 6 months
Reagent 3	2 mmol/L Standard	2 mL × 2 vials	-20℃ , shading light, 6 months
	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 (650-670 nm, optimum wavelength: 660 nm), Micropipettor, 37°C incubator.

Reagents

Double distilled water, 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

1. When adding reagent 2, suck and beat with micropipettor repeatedly to ensure the color system mix fully.
2. Prevent the formulation of bubbles when the sample is transferred into the microplate.

Pre-assay preparation

▲Reagent preparation

Bring all reagents to room temperature before use.

▲Sample preparation

1. Serum (plasma): Detect the sample directly.
2. Tissue: Weigh the tissue accurately. Add 60% ethanol (self-prepared) in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break cells fully. Then centrifuge at 10000 g for 10 min at 4°C and collect the supernatant for measurement.

▲Sample requirements

The samples should not contain SDS, Tween20, NP-40, Triton X-100 and other detergents, and should not contain DTT, 2-merhydryl ethanol and other reducing reagents.

▲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 (0.23-2 mmol Trolox Equiv. /L).

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat lung tissue homogenate	1
Molt4 cellar supernatant	1
Human urine	8-10
Mouse serum	1
Human serum	1
Human saliva	1

Note: The diluent is 60% ethanol.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	660 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

▲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.

▲Operating steps

The preparation of standard curve

Dilute 2 mmol/L standard solution with 60% ethanol (self-prepared) to a serial concentration. The recommended dilution gradient is as follows: 0, 0.4, 0.8, 1.2, 1.4, 1.6, 1.8, 2 mmol/L.

The measurement of samples

1. Sample well: Add 10 μL of sample to the sample well.
   Standard well: Add 10 μL of standard with different concentration to the standard well.
2. Add 200 μL of reagent 1 to each well.
3. Measure the OD values of each well at 660 nm with microplate reader, recorded as A_1.
4. Add 20 μL of reagent 2 to each well, repeatedly suck and beat for 5-6 times.
5. Incubate at 37°C for 5 min. Measure the OD values of each well at 660 nm with microplate reader, recorded as A_2. $\Delta A=A_2-A_1$

▲Operation table

	Standard well	Sample well
Sample (μL)		10
Standards with different concentrations (μL)	10	
Reagent 1 (μL)	200	200
Measure the OD values of each well at 660 nm with microplate reader, recorded as A_1.		
Reagent 2 (μL)	20	20
Repeatedly suck and beat for 5-6 times. Incubate at 37°C for 5 min. Measure the OD values of each well at 660 nm with microplate reader, recorded as A_2. $\Delta A=A_2-A_1$.		

▲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.
The standard curve is: y= ax + b.

1. Liquid sample (Trolox is used as a reference substance for total antioxidant status):

$$TAS\ (mmol\ Trolox\ Equiv.\ /L) = (\Delta A_{Blank} - \Delta A_{Sample} - b) \div a \times f$$

2. Tissue and cells sample:

$$TAS\ (mmol\ Trolox\ Equiv.\ /kg\ wet\ weight) = (\Delta A_{Blank} - \Delta A_{Sample} - b) \div a \div (m \div v) \times f$$

Note:

y: $\Delta A_{Blank} - \Delta A_{Standard}$ (ΔA_{Blank} is ΔA when the standard concentration is 0);
x: The concentration of Standard;
a: The slope of standard curve;
b: The intercept of standard curve;
ΔA_{Sample}: The OD value of sample (A_2-A_1);
m: The weight of tissue sample (g);
V: The volume of added homogenate (mL);
f: Dilution factor of sample before test;

▲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

Appendix I Performance characteristics			
Detection range	0.23-2 mmol Trolox Equiv. /L	Average intra-assay CV (%)	4.6
Sensitivity	0.23 mmol Trolox Equiv. /L	Average inter-assay CV (%)	7.0
Average recovery rate (%)	99		

▲Example analysis

For human serum, take human serum sample and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.4056 x + 0.0342, the OD value of the sample (A_1) is 0.08, the OD value of the sample (A_2) is 0.777, $\Delta A_{Sample} = A_2 - A_1 = 0.697$, ΔA_{Blank} is 1.196, and the calculation result is:

TAS (mmol Trolox Equiv. /L) = (1.196–0.697 -0.0342) ÷ 0.4056

=1.14 mmol Trolox Equiv. /L