Technical Manual

Total Antioxidant Status (TAS) Colorimetric Assay Kit

- **Catalogue Code: MAES0198**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.23-2 mmol Trolox Equiv. /L

Sensitivity:

0.23 mmol Trolox Equiv. /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

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.

3. 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.

4. Detection Principle

ABTS is oxidized to green ABTS•+ 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•+ 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.

5. Kit Components & Storage

Item	Specification	Storage
Buffer Solution	30 mL × 1 vial	-20°C, 6 months
Chromogenic Agent	5 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Standard (2 mmol/L)	2 mL × 2 vials	-20°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- incubator
- Microplate Reader (650-670 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- 60% Ethanol

6. Assay Notes:

1. When adding chromogenic agent, 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.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation

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.

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. 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 60% Ethanol (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 (0.23-2 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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 660 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 2 mmol/L standard solution with 60% ethanol 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 buffer solution 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 chromogenic agent 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	
Buffer solution (μL)	200	200
Measure the OD values of each well at 660 nm with microplate reader, recorded as A_1.		
Chromogenic agent (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. $\Delta A=A_2-A_1$.		

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

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

2. Tissue sample:

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

y: $\triangle_{Standard} - \triangle_{Blank}$ ($\triangle_{Blank}$ is the OD value 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.

12. Performance Characteristics

Detection Range	0.23-2 mmol Trolox Equiv. /L
Sensitivity	0.23 mmol Trolox Equiv. /L
Average recovery rate (%)	99
Average inter-assay CV (%)	7
Average intra-assay CV (%)	4.6

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, ΔA_{Sample} = A2 - A1 =0.697, ΔA_{Blank} is 1.196, and the calculation result is:

$$\text{TAS (mmol Trolox Equiv./L)} = (1.196 - 0.697 - 0.0342) \div 4056$$

$$= 1.14 \text{ mmol Trolox Equiv. /L}$$

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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.

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Notes:

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