Technical Manual

Total Oxidant Status (TOS) Colorimetric Assay Kit

- Catalogue Code: MAES0199
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

2.5-100 µmol H_2O_2 Equiv./L

Sensitivity:

2.5 µmol H_2O_2 Equiv./L

Storage:

2-8°C for 3 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 3 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. Under natural physiological conditions, the increase and decrease of oxidative molecules cannot be prevented, and the oxidation/antioxidant balance shifts to the oxidation state, which will lead to a variety of diseases related to oxidative stress. The concentrations of different oxidants in serum (or plasma) can be measured individually by experiment, but the process is time consuming, costly and technically complex. Since it is not practical to measure the different oxidant molecules individually and their oxidation effects are additive, the total oxidation state (TOS) of the sample determined is needed.

3. Intended Use

The kit is used for the determination of total antioxidant status (TAS) in tissue, serum, plasma and other liquid samples.

4. Detection Principle

Under acid conditions, the oxidizing material in the sample can oxidize Fe2+ to Fe3+, which binds highly with xylenol orange to produce a blue-purple complex. When the pH of solution is in the range of 2-3, its maximum absorption wavelength is around 590 nm, and the color depth is proportional to the content of oxidation substances in a certain concentration and a certain time, so as to indirectly calculate the total oxidation state of the sample.

5. Kit Components & Storage

Item	Specification	Storage
Chromogenic Agent	24 mL × 1 vial	2-8°C, 3 months
Substrate	6 mL × 1 vial	2-8°C, 3 months
H_2O_2 Standard (200μmol/L)	1 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- incubator
- Centrifuge
- Microplate Reader (580-590 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)

6. Assay Notes:

1. It is recommended to aliquot chromogenic agent into smaller quantities in EP tube before use to prevent contamination.

2. Substrate should be sealed in time after use and should not be exposed to air for a long time.

3. 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

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 Normal saline (0.9% NaCl), 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 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 (2.5-100 μmol H_2O_2 Equiv./L).

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

Sample Type:	Dilution Factor:
Human serum	1
Porcine serum	1
Horse serum	1
Cynomolgus macaques serum	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 590 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 200 μmol/L H_2O_2 standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 μmol/L.

The measurement of samples

1. **Standard well:** Add 20 μL of standard with different concentration to the standard well.
   Sample well: Add 20 μL of sample to the sample well.

2. Add 200 μL of chromogenic agent to each well.

3. Mix fully with microplate reader for 5 s and measure the OD values of each well at 590 nm with microplate reader, recorded as A_1.

4. Add 50 μL of substrate to each well.

5. Mix fully with microplate reader for 5 s and incubate at 37°C for 5 min. Measure the OD values of each well at 590 nm with microplate reader, recorded as A_2. $\Delta A=A_2-A_1$.

Operation Table

	Standard well	Sample well
Standards with different concentrations (μL)	20	
Sample (μL)		20
Chromogenic agent (μL)	200	200
Mix fully with microplate reader for 5 s and measure the OD values of each well at 590 nm with microplate reader, recorded as A_1.		
Substrate (μL)	50	50
Mix fully with microplate reader for 5 s and incubate at 37°C for 5 min. Measure the OD values of each well at 590 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. Serum and other liquid sample:

$$\text{TOS } (\mu\text{mol } H_2O_2 \text{ Equiv./L}) = \frac{\Delta A_{590} - b}{a} \times f$$

2. Tissue sample:

$$\text{TOS } (\mu\text{mol } H_2O_2 \text{ Equiv./gprot}) = \frac{\Delta A_{590} - b}{a} \div C_{pr} \times f$$

y: $OD_{Standard}$ – OD_{Blank} (OD_{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_{590}: OD_{Sample} – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics:

Detection Range	2.5-100 µmol H_2O_2 Equiv./L
Sensitivity	2.5 µmol H_2O_2 Equiv./L
Average inter-assay CV (%)	3.5
Average intra-assay CV (%)	2.3

Analysis

For human serum, take 20 µL to the sample wells and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.007 x - 0.0146, the ΔA value of the sample is 0.142, the average ΔA value of the blank is 0.098, the absolute ΔA value of the sample: ΔA_{590}=0.142-0.098 =0.044, and the calculation result is:

$$\text{TOS}\ (\mu\text{mol } H_2O_2 \text{ Equiv./L}) = (0.044 + 0.0146) \div 0.007$$

$$= 8.37\ \mu\text{mol } H_2O_2 \text{ Equiv./L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid 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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