Total Oxidant Status (TOS)

Colorimetric Assay Kit

Catalog No: E-BC-K802-M

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 2.5 μmol H_2O_2 Equiv./L

Detection range: 2.5-100 μmol H_2O_2 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 (TOS) in tissue, serum and other liquid 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. 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.

▲Detection principle

Under acid conditions, the oxidizing material in the sample can oxidize Fe^{2+} to Fe^{3+}, 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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Chromogenic Agent	24 mL × 1 vial	2-8°C , shading light, 3 months
Reagent 2	Substrate	6 mL × 1 vial	2-8°C , shading light, 3 months
Reagent 3	1 mmol/L H_2O_2 Standard	1 mL × 1 vial	2-8°C , shading light, 3 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 (580-590 nm, optimum wavelength: 590 nm), Micropipettor, 37°C incubator.

Reagents

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

▲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. It is recommended to aliquot reagent 1 into smaller quantities in EP tube before use to avoid contamination.
2. Reagent 2 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.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of 200 μmol/L standard solution:
   Dilute 1 mmol/L H_2O_2 standard with double distilled water for 5 times.
   Prepare the fresh solution before use.

▲Sample preparation

1. Serum: Detect the sample directly.

2. Tissue: Weigh the tissue accurately. Add normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) 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. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲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 (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 of is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	590 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 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 reagent 1 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 reagent 2 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
Reagent 1 (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.		
Reagent 2 (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. $\Delta A=A_2-A_1$.		

▲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. Serum and other liquid sample:

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

2. Tissue sample:

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

Note:

y: $\Delta A_{Standard} - \Delta A_{Blank}$ (ΔA_{Blank} is the 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_{590}: $\Delta A_{Sample} - \Delta A_{Blank}$ (ΔA_{Blank} is the ΔA when the standard concentration is 0);
f: Dilution factor of sample before test;
C_{pr}: Protein concentration of sample, gprot/L.

▲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 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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

▲Example 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:

TOS (μmol H_2O_2 Equiv./L) = (0.044+0.0146)÷0.007 =8.37μmol H_2O_2 Equiv./L