Technical Manual

Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit (FRAP Method)

- Catalogue Code: MAES0150
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.049-2.5 mmol/L

Sensitivity:

0.049 mmol/L

Storage:

2-8°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.

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2. Background

There are two kinds of antioxidant systems in the body. One is enzyme antioxidant system, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Another group of non-enzymatic antioxidant systems includes uric acid, vitamin C, vitamin E, glutathione, bilirubin, alpha-lipoic acid, and carotenoids. Antioxidant capacity is considered to be the cumulative effect of all antioxidants in the blood and body fluids.

3. Intended Use

The kit is used to measure the total antioxidant capacity (T-AOC) in serum, plasma, tissue homogenate, cell, cell culture supernatant, saliva and urine samples.

4. Detection Principle

Fe^{3+}-TPTZ can be reduced by antioxidants and produce blue Fe^{2+}-TPTZ under acid condition. The antioxidant capacity of sample can be calculated by detection the absorbance value at 593 nm.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	20 mL × 1 vial	2-8⁰C, 6 months
TPTZ Solution	2 mL × 1 vial	2-8⁰C, 6 months, avoid direct sunlight
Substrate Solution	2 mL × 1 vial	2-8⁰C, 6 months, avoid direct sunlight
$FeSO_4 \cdot 7H_2O$ Standard	200 mg × 1 vial	2-8⁰C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (590-600 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. Reagents which are blue or approximate blue in acidic condition will make influence on the detection result should be prevented as possible.

2. Prepare the fresh $FeSO_4$ solution before use.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **FRAP working solution:** Prepare the needed amount of FRAP working solution according to the ratio of buffer solution: TPTZ solution: substrate solution = 10: 1: 1. Prepare the fresh solution before use. The prepared FRAP working solution should be used within 2 hours.

3. Preparation of **$FeSO_4$ solution (100 mM):** Weigh 27.8 mg of $FeSO_4 \cdot 7H_2O$ standard accurately and dissolve with 1 mL of double distilled water. Prepare the fresh solution before use.
   Note: Fe^{2+} is easily oxidized to Fe^{3+}, the color will change from light green to light yellow. Please discard the solution if it color is yellow.

8. Sample Preparation

Sample requirements: The sample should not contain reducing reagents such as DTT, 2-Hydroxy-1-ethanethiol etc.

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. 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°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 1000-2000 g for 10 min at 4°C. 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°C for a month.

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

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4. Saliva:
Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4°C. Take the supernatant and preserve it on ice for detection. If not detected on the same day, the saliva can be stored at -80°C for a month.

5. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, 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 (2×10^6): PBS (0.01 M, pH 7.4) including 5.6 mM sucrose (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

6. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) including 5.6 mM sucrose (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 to preserve it on ice for 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 (0.049-2.5 mmol/L).

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

Sample Type:	Dilution Factor
Human serum	1
Human saliva	1
Human urine	1
Cellular supernatant	1
HepG2 cells homogenization	1
5% Mouse liver tissue homogenization	1
10% Epipremnum aureum tissue homogenization	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: 593 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 $FeSO_4$ solution (100 mmol/L) with distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.3, 0.6, 0.9, 1.2, 1.8, 2.1, 2.5 mmol/L.

The measurement of samples

1. **Standard well:** Take 5 μL of standard solution with different concentrations to the wells.
   Sample well: Take 5 μL of sample to the wells.

2. Add 180 μL of FRAP working solution into the wells of step 1.

3. Incubate at 37 ºC for 3~5 min, then measure the OD values of each well with Microplate reader at 593 nm.

Operation Table

	Standard well	Sample well
Standard solution with different concentrations (μL)	5	
Sample (μL)		5
FRAP working solution (μL)	180	180
Incubate at 37 ºC for 3~5 min, then measure the OD values of each tube with Microplate reader at 593 nm		

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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 (plasma) and other liquid sample:

T-AOC（mmol/L）= $(\Delta A_{593}$ - b) ÷ a × f

2. Tissue and cell sample:

T-AOC（mmol/gprot）= $(\Delta A_{593}$ - b) ÷ a × f ÷ C_{pr}

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_{593}: $OD_{Sample.}$ – OD_{Blank}
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

12. Performance Characteristics

Detection Range	0.049-2.5 mmol/L
Sensitivity	0.049 mmol/L
Average recovery rate (%)	103
Average inter-assay CV (%)	8.1
Average intra-assay CV (%)	3.9

Analysis

Take 5 μL of human serum, carry the assay according to the operation table.

The results are as follows:
standard curve: y = 0.30304x +0.001, the average OD value of the sample is 0.2491, the average OD value of the blank is 0.0572, and the calculation result is:

T-AOC (mmol/L) = (0.2491-0.0572-0.001) ÷ 0.30304 ×1 = 0.63 (mmol/L)

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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