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

Catalog Number EEA024 (96 tests)

Rev 1.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

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

There are two kinds of antioxidant systems in the body. One is the 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.

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

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Buffer Solution	20 mL
TPTZ Solution	2 mL
Substrate Solution	2 mL
$FeSO_4 \cdot 7H_2O$ Standard	200 mg
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 593 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

- The sample should not contain reducing reagents such as DTT, 2-Hydroxy-1-ethanethiol, etc or detergents (Tween, Triton, NO-40, etc).

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for detection.

Urine sample: Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃. Take the supernatant to preserve it on ice for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃.
- Take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month

Cells:

- Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) 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 (10^6): PBS (0.01 M, pH 7.4) (μL) =1: 300-500.
- Homogenize cells by an ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min) or homogenizer instrument (60 Hz 90s) on ice.
- Centrifuge at 10000 g for 10 min at 4℃, then take the supernatant and preserve it on ice for detection.

Meanwhile, determine the protein concentration of supernatant (Catalog No. 23227). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for 1 month.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and 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).

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. Mix fully and store away from light. Prepare the fresh solution before use and use within 2 hours of preparation.

Preparation of 100 mM $FeSO_4$ solution

Weigh 27.8 mg of $FeSO_4 \cdot 7H_2O$ Standard accurately and dissolve with 1 mL of distilled water. Prepare the fresh solution before use.

Note: Fe^{2+} is easily oxidized to Fe^{3+}, where the color will change from light green to light yellow. Please discard the solution if the color is yellow.

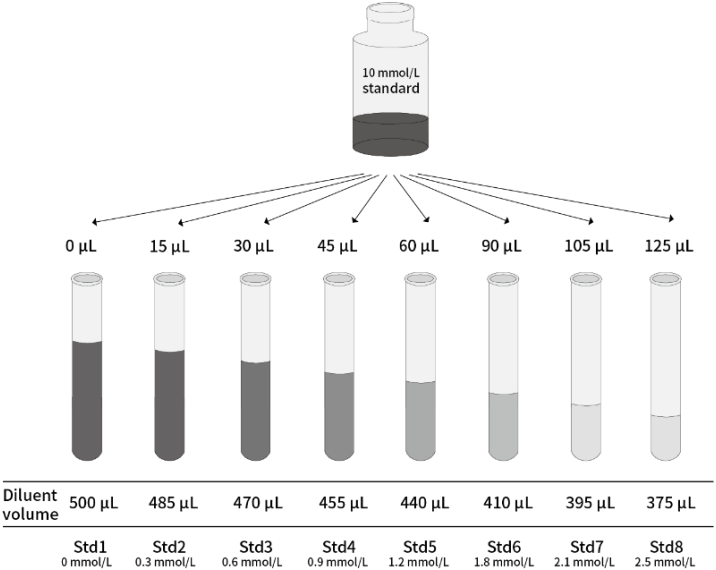
Preparation of 10 mM $FeSO_4$ solution

Dilute 1 part of 100 mM $FeSO_4$ solution with 9 part of distilled water and mix fully.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

Dilute 10 mmol/L $FeSO_4$ solution 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.



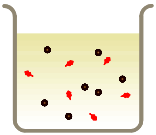
The key point of the assay

- Reagents which are blue or near blue in acidic condition will influence the detection results and should be avoided.
- High concentration of Fe^{3+} salt or Fe^{2+} salt in samples may interfere the result, because they will inhibit the interference of endogenous substances in samples under acid conditions. Total concentration of Fe^{3+} salt or Fe^{2+} in serum (plasma) is always lower than 10 μM, which will not interfere the FRAP detection. Small amount of metal chelating agent in samples will not affect the detection.
- Substances which may affect the oxidation-reduction reaction (e.g., DTT and mercaptoethanol) and detergent (e.g., Tween, Triton and NP-40) cannot be added into

samples.

- It is recommended to store samples at -80 ℃ if the detection cannot be operated timely. The detection result will not change obviously within 1 month.
- TPTZ solution is irritant for humans, please wear lab-gown and gloves during the operation.

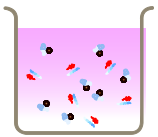
Assay Protocol



1. **Add sample and standard**

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

b. **Sample well:** Take 5 μL of sample to the wells.



2. **Add substrate**

a. Add 180 μL of FRAP working solution to each well.

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

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Serum (plasma) and other liquid sample:

$$\text{T-AOC (mmol/L)} = (\Delta A_{593} - b) \div a \times f$$

Tissue and cells sample:

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

[Note]

y: The absolute OD value of standard;

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)

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

Take 5 μL of human serum, carry the assay according to the operation steps. The results are as follows:

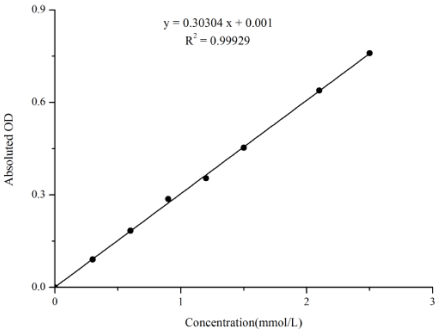
standard curve: y = 0.30304x +0.001, the average OD value of the sample is 0.2491, the average OD value of the blank is 0.0572, and the calculation result is:

$$\text{T-AOC (mmol/L)} = (0.2491 - 0.0572 - 0.001) \div 0.30304 \times 1 = 0.63 \text{ (mmol/L)}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–2.5 mmol/L standard.

Concentration (mmol/L)	OD at 593 nm	Standard Curve
2.50	0.829	
2.10	0.708	
1.80	0.618	
1.20	0.423	
0.90	0.356	
0.60	0.253	
0.30	0.160	
0.00	0.070	

- Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.10	1.0	1.7
%CV	9.5	8.3	6.4

CV = Coefficient of Variation

- Intra-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.10	1.0	1.7
%CV	6.2	3.3	2.1

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum sample without dilutions.

Sample Type	Range (**mmol/L**)	Average (**mmol/L**)
Human serum	0.5-0.9	0.63
Human urine	1.9-3.3	2.23

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (mmol/L)	0.1	1	1.7
Observed Conc. (mmol/L)	0.11	1.01	1.66
Recovery rate (%)	110	101	97

- Recommended 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												

- Sensitivity

The analytical sensitivity of the assay is 0.049 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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