Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit

Catalog Number EEA022 (96 tests)

Rev 2.0

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

The kit is used for the determination of total antioxidant capacity (T-AOC) in serum, plasma, whole blood, tissue, cell and culture supernatant samples. There are two kinds of antioxidant system, one is enzyme antioxidant system, including superoxide dismutase (SOD),) catalase (CAT), glutathione peroxidase (GSH-Px). The other is non-enzymatic antioxidant systems, including uric acid, vitamin C, vitamin E, glutathione, bilirubin, α-lipoic acid, carotenoid. Antioxidant capacity is thought to be the cumulative effect of all antioxidants in blood and body fluids.

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflect the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 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	12 mL
Chromogenic Agent	Powder ×2 vials
Ferric Salt Stock Solution	0.4 mL
Ferric Salt Diluent	8 mL
Stop Solution	1.25 mL ×2 vials
Clarificant	1.25 mL ×2 vials
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 520 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 DTT, 2-mercaptoethanol and other reducing agents.
- The supernatant of sample preparation after centrifugation must be clarified, otherwise centrifuge again.

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.

Whole blood sample:

- Collect the fresh blood to the test tube containing anticoagulant ($V_{anticoagulant}$:V_{blood}=1:9), mix gently.
- The sample can be stored at 2-8℃ for 1-2 days.

Cell culture supernatant: Detect the cell culture supernatant directly. If there is turbidity, centrifuge at 3100 g for 10 min, take the supernatant and preserve it on ice for detection. If not detected on the same day, stored the serum at -80℃, which can be stored for a month.

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) for 1 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 (0.62-190.43 U/mL). The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human urine	1-2
10% Rat liver tissue homogenization	1
10% Epipremnum aureum tissue homogenization	1
HepG2 cells	1
HepG2 cells culture supernatant	1

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

Preparation of Chromogenic Agent working solution

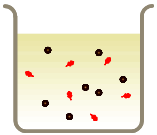
Dissolve a vial of powder with 20 mL of distilled water fully (It can be dissolved by incubating in 80-90℃ water bath). It can be used after cooling to room temperature. Store at 2-8 ℃ for 7 days.

Preparation of Ferric Salt Stock working solution

Prepare fresh solution before use by mixing 19 parts of Ferric Salt Diluent with 1 part of Ferric Salt Stock Solution. Store at 2-8 ℃ for 2 days.
Note: Clarificant will be freeze in cold weather, dissolve by incubating in 37 ℃ water bath until clarification before experiment.

Assay Procedure

For serum (plasma) and other liquid samples



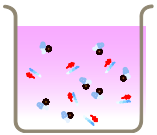
1. Add sample and control

a. **Sample tube:** Add 100 μL of Buffer Solution to 1.5 mL EP tube

Control tube: Add 100 μL of Buffer Solution to 1.5 mL EP tube

b. **Sample tube**: Add 10 μL of sample to the tube.

Control tube: Add nothing.



2. Add substrate

a. Add 200 μL of chromogenic agent working solution and 50 μL of Ferric Salt Stock working solution to sample tube and control tube.

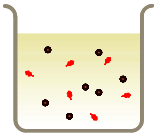
b. Mix fully and incubate the tubes at 37℃ for 30 min

c. Add 10 μL of stop solution to sample tube and control tube.

d. Add 10 μL of sample to the control tube.

e. Mix fully and stand for 5 min at room temperature. Pipette 300 μL to each well of the 96 well plate and measure the OD value of each well at 520 nm with microplate reader.

For tissue and cells samples



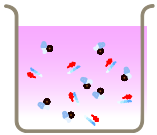
1. Add sample and control

a. **Sample tube:** Add 100 μL of Buffer Solution to 1.5 mL EP tube

Control tube: Add 100 μL of Buffer Solution to 1.5 mL EP tube

b. **Sample tube:** Add 10 μL of sample to the tube.

Control tube: Add nothing.



2. Add substrate

a. Add 200 μL of chromogenic agent working solution and 50 μL of Ferric Salt Stock working solution to sample tube and control tube.

b. Mix fully and incubate the tubes at 37℃ for 30 min.

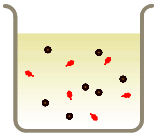
c. Add 20 μL of stop solution to sample tube and control tube.

d. Add 10 μL of sample to the control tube.

e. Add 20 μL of clarificant to sample tube and control tube

f. Mix fully and stand for 5 min at room temperature. Pipette 300 μL to each well of the 96 well plate and measure the OD value of each well at 520 nm with microplate reader.

For whole blood samples



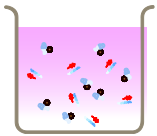
1. Add sample and control

a. **Sample tube:** Add 100 μL of Buffer Solution to 1.5 mL EP tube

Control tube: Add 100 μL of Buffer Solution to 1.5 mL EP tube

b. **Sample tube**: Add 10 μL of sample to the tube.

Control tube: Add nothing.



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b. Mix fully and incubate the tubes at 37℃ for 30 min

c. Add 20 μL of stop solution to sample tube and control tube.

d. Add 10 μL of sample to the control tube.

e. Mix fully and stand for 5 min at room temperature. Pipette 300 μL to each well of the 96 well plate and measure the OD value of each well at 520 nm with microplate reader.

Calculation

Serum (plasma), whole blood and other liquid sample:

Unit definition: At 37 ℃, the OD value of the reaction system was increased 0.01 by 1 mL of sample per minute is defined as a unit of total antioxidant capacity.

$$\underset{(U/mL)}{T-AOC} = \frac{\Delta A}{0.01} \div 30^{*} \times \frac{V_1}{V_2} \times f$$

Tissue and cells sample:

Unit definition: At 37 ℃, the OD value of the reaction system was increased 0.01 by 1 mg of protein per minute is defined as a unit of total antioxidant capacity.

$$\underset{(U/mgprot)}{T\text{-}AOC} = \frac{\Delta A}{0.01} \div 30^{*} \times \frac{V_1}{V_2} \times f \div C_{pr}$$

[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: $OD_{Sample} - OD_{Control.}$

*: The reaction time, 30 min.

V_1: The total volume of reaction, mL

V_2: The volume of sample added to the reaction, mL

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 10 μL of human serum sample and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.081, the average OD value of the control is 0.020, and the calculation result is:

$$\underset{(U/mL)}{T-AOC} = \frac{0.081\text{-}0.020}{0.01} \div 30 \times \frac{0.37}{0.01} = 7.52\ U/mL$$

- Intra-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 (U/mL)	3.50	64.50	138.50
%CV	5.1	4.7	4.6

CV = Coefficient of Variation

- Inter-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 (U/mL)	3.50	64.50	138.50
%CV	5.3	5.7	5.8

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum samples without dilutions.

Sample Type	Range (**U/mL**)	Average (**U/mL**)
Human serum	11.02-15.42	13.22

- 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 96%.

	Standard 1 (low conc.)	Standard 2 (middle conc.)	Standard 3 (high conc.)
Expected Conc.（U/mL）	22.5	84.6	164.5
Observed Conc.（U/mL）	22.3	80.4	154.6
Recovery rate (%)	99	95	94

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'
[Note]: S1-S48, sample wells; S1'-S48', control wells												

- Sensitivity

The analytical sensitivity of the assay is 0.62 (U/mL). 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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