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

Catalog No: E-BC-K225-M

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.049 mmol/L

Detection range: 0.049-2.5 mmol/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 to measure the total antioxidant capacity (T-AOC) in serum, plasma, tissue homogenate, cell, cell culture supernatant, saliva and urine samples.

## ▲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.

## ▲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.

## Kit components & Storage

| Item | Component | Specification | Storage |
|---|---|---|---|
| Reagent 1 | Buffer Solution | 20 mL × 1 vial | 2-8ºC, 6 months |
| Reagent 2 | TPTZ Solution | 2 mL × 1 vial | 2-8ºC, 6 months, shading light |
| Reagent 3 | Substrate Solution | 2 mL × 1 vial | 2-8ºC, 6 months, shading light |
| Reagent 4 | $FeSO_4 \cdot 7H_2O$ Standard | 200 mg × 1 vial | 2-8ºC, 6 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 (590-600 nm), Balance, Incubator, Micropipettor

### Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

### 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 points of the assay

1. Reagents which are blue or approximate blue in acidic condition will make influence on the detection result should be avoided as possible.
2. 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 condition. 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.
3. 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.
4. It is recommended to store samples at -80ºC if the detection cannot be operated timely. The detection result will not change obviously within 1 month.
5. Reagent 2 is irritant for humans, please wear lab-gown and gloves during the operation.

# Pre-assay preparation

## ▲Reagent preparation

1. Preparation of FRAP working solution:
   Prepare the needed amount of FRAP working solution according to the ratio of reagent 1: reagent 2: reagent 3 = 10: 1: 1. Mix fully and store with shading light. Use the FRAP working solution within 2 hours. Prepare the fresh solution before use.
2. Preparation of 100 mM $FeSO_4$ solution:
   Weigh 27.8 mg of reagent 4 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.

## ▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

### Sample requirements

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

## ▲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 (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).

| Assay protocol | |
|---|---|
| Ambient temperature | 25-30℃ |
| Optimum detection wavelength | 593 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, blank well; B-H, standard wells; S1-S80, sample wells.

## ▲Operating steps

### The preparation of standard curve

Dilute 100 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 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.

Note: Prevent the formulation of bubbles when the liquid is transferred into the microplate.

## ▲Operation table

| | Standard well | Sample well |
|---|---|---|
| $Fe_2SO_4$-$7H_2O$ 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. | | |

## ▲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. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

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

2. Tissue and cells sample:

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

**Note:**

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.

$\Delta A_{593}$: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

$C_{pr}$: Concentration of protein in 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 6 months.
4. Do not use components from different batches of kit.

# Appendix I Performance characteristics

| Appendix I Performance characteristics | | | |
|---|---|---|---|
| Detection range | 0.049-2.5 mmol/L | Average intra-assay CV (%) | 3.9 |
| Sensitivity | 0.049 mmol/L | Average inter-assay CV (%) | 8.1 |
| Average recovery rate (%) | 103 | | |

## ▲Example 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)

# Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

## ▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . 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℃ for a month.

## ▲Plasma

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

## ▲Urine

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

## ▲Saliva

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

### ▲Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (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 to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

### ▲Cells

Collect the cells and wash the cells with homogenization medium 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 ($10^6$): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 1500 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

**Note:**

1. Homogenized medium: PBS (0.01 M, pH 7.4) including 5.6 mM sucrose.
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 $mm^3$), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 10 min).

## ▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.