

# Total antioxidant capacity (T-AOC) Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Cat No:** BC1310

**Size:** 50T/48S

## Components:

**Extract solution:** Liquid 50 mL×1. Storage at 4°C, precool before use.

**Reagent I:** Liquid 35 mL×1. Storage at 4°C.

**Reagent II:** Liquid 20 mL×1. Storage at 4°C in shadow.

**Reagent III:** Liquid 5 mL×1. Storage at 4°C in shadow.

**Standard:** Powder×1, 10 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . Working solution: add 0.9 mL of distilled water and 20  $\mu\text{L}$  of concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to forms 40  $\mu\text{mol/mL}$   $\text{FeSO}_4$  standard solution.

Solution mixture (prepare when the solution will be used): Reagent I : Reagent II: Reagent III= 7:1:1, incubate at 37°C before use.

## Product Description:

This kit is used to detect the total antioxidant levels of antioxidants and antioxidant enzymes in the samples. It is mainly used in the study of biological, medical and pharmaceutical studies to detect the total antioxidant capacity of antioxidant solutions.

In acid environment,  $\text{Fe}^{3+}$ -TPTZ are reduced to blue  $\text{Fe}^{2+}$ -TPTZ. The color reaction reflects the total antioxidant capacity

## Reagents and Equipment Required but Not Provided:

Spectrophotometer, constant temperature water bath, low temperature centrifuge, 1 mL glass cuvette and distilled water.

## Procedure:

### I. Sample preparation:

#### 1. Serum, plasma, saliva or urine samples

Plasma (anticoagulation with heparin or sodium citrate, avoid using EDTA), centrifuge at 5000 rpm/min for 10 min, take supernatant for test. Take serum, saliva or urine samples for direct determination. Also you can store at -80°C and detect within 30 days.

#### 2. Cells or tissue sample

Take 1-2 million cells or 0.1 g of tissue, add 1.0 mL of Extract solution. Use homogenate or ultrasound to fully break up cells and release antioxidant, centrifuge at 10000 r/min and 4°C for 5 min, take supernatant for test. Measure the concentration of protein if needed.

### II. Determination procedure:

- Dilute 40  $\mu\text{mol/mL}$   $\text{FeSO}_4$  standard solution to 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125  $\mu\text{mol/mL}$ , take 500  $\mu\text{L}$  of standard solution (distilled water for blank control), add to 500  $\mu\text{L}$  of Reagent II. Mix thoroughly for 10 min, detect the absorbance in 593 nm, calculate  $\Delta A = A_S - A_B$ . ( $A_S$ : standard solution tube,  $A_B$ : blank control tube.) The final concentration of  $\text{Fe}^{2+}$  is 0.05、0.025、0.0125、0.00625、0.003125、0.00156  $\mu\text{mol/mL}$ .
- Preheat the spectrophotometer 30 min, adjust wavelength to 593 nm and set zero with distilled water.
- Add reagents with the following list:

Reagent Name	Blank tube ( $A_B$ )	Test tube ( $A_T$ )
Solution mixture ( $\mu\text{L}$ )	900	900
Sample ( $\mu\text{L}$ )		30
Double distilled water ( $\mu\text{L}$ )	120	90
Mix thoroughly and react for 10 min, set zero with distilled water, detect the absorbance in 593nm. Calculate $\Delta A' = A_T - A_B$ . <b>(Note: The blank tube just need to be tested once or twice in every experiment)</b>		

### III. Calculation:

- Create standard curve

Take the  $\text{Fe}^{2+}$  final concentration as X-axis,  $\Delta A$  as Y-axis, create standard curve, get linear regression equation  $y = kx + b$ , take  $\Delta A'$  into the equation to get x ( $\mu\text{mol/mL}$ ).

- Unit definition: the sample antioxidant capacity is indicated by the standard liquid ion concentration required for the same absorbance change ( $\Delta A$ ).

#### A. Protein concentration:

Total antioxidant capacity ( $\mu\text{mol/mg prot}$ ) =  $x \times V_{rv} \div (V_s \times C_{pr}) = 34 \times x \div C_{pr}$

#### B. Sample weight

Total antioxidant capacity ( $\mu\text{mol/g weight}$ ) =  $x \times V_{rv} \div (V_s \div V_{sv} \times W) = 34 \times x \div W$

#### C. Cell amount

Total antioxidant capacity ( $\mu\text{mol}/10^4\text{cell}$ ) =  $x \times V_{rv} \div (V_s \times V_{sv} \div n) = 34 \times x \div n$

#### D. Solution volume

Total antioxidant capacity ( $\mu\text{mol/mL}$ ) =  $x \times V_{rv} \div V_s = 34 \times x$

$V_{rv}$ : total reaction volume, 1.02 mL;

$V_s$ : sample volume, 0.03 mL;

$V_{sv}$ : extraction volume, 1 mL;

$W$ : sample weight, g;

$C_{pr}$ : sample protein concentration, mg/mL;

$n$ : cell amount, unit based on  $10^4$  (ten thousand).

### Note:

- Reagent II is irritated to human body, please wear lab clothes and latex gloves.

2. The samples should not be appear blue under acidic condition, or it will interference sample result of the kit.
3. Detergent such as Tween, Triton, NP-40 and reductants such as DTT, mercapto ethanol should not be added in the sample.
4. If the absorbance value determined by the sample is beyond the standard curve range, the sample should be diluted or concentrated properly before determination.
5. The kit should be store at 2-8°C.

#### Examples:

1. Add 0.1g shamrock to 1mL extract solution and grind thoroughly on ice, take supernatant, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate:  $\Delta A = A(T) - A(B) = 0.909 - 0.148 = 0.761$ , standard curve:  $y = 21.056x - 0.0087$ , calculate  $x = 0.037$ , according with mass of sample to calculate Total antioxidant capacity ( $\mu\text{mol/g mass}$ )  $= 34 \times x \div W = 34 \times 0.037 \div 0.1 = 12.85 \mu\text{mol/g mass}$ .

#### Recent Product citations:

[1] An W, Zhang Y, Zhang X, et al. Ocular toxicity of reduced graphene oxide or graphene oxide exposure in mouse eyes[J]. Experimental eye research, 2018, 174: 59-69.

[2] Zhang S, He Y, Sen B, et al. Alleviation of reactive oxygen species enhances PUFA accumulation in Schizochytrium sp. through regulating genes involved in lipid metabolism[J]. Metabolic engineering communications, 2018, 6: 39-48.

[3] Liu S, You L, Zhao Y, et al. Wild Lonicera caerulea berry polyphenol extract reduces cholesterol accumulation and enhances antioxidant capacity in vitro and in vivo[J]. Food Research International, 2018, 107: 73-83.

[4] Z Zhang, H Liu, C Sun, et al. A C2H2 zinc-finger protein OsZFP213 interacts with OsMAPK3 to enhance salt tolerance in rice. Journal of plant Physiology. October 2018; 100-110.(IF7.394)

[5] Esmail S. Kakey, Amez A. Ismael. Evaluation of Oxidative Stress Status in Aged Human in relation to some Diseases. International Conference on Pure and Applied Sciences. August 2018

#### References:

[1] Pellegrini N, Serafini M, Salvatore S, et al. Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different in vitro assays[J]. Molecular nutrition & food research, 2006, 50(11): 1030-1038.

#### Related Products:

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|---------------|--|
| BC1320/BC1325 | Hydroxyl Radical Scavenging Capacity Assay Kit |
| BC1330/BC1335 | Plant Flavonoids Assay Kit                     |
| BC1340/BC1345 | Plant Total Phenol (TP) Assay Kit              |

