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Ver.250201

## **Total antioxidant capacity (T-AOC) Assay Kit**

BC3305-02 (100 Tests/96 Samples)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

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

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

## Kit components

Reagent	Volume	Storage
Extraction Solution	110mL	4°C, Precool before use
Reagent I	20mL	4°C
Reagent II	5mL	4°C, Protect from light
Reagent III	2mL	4°C, Protect from light
Standard	20mg ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ )	

### Note:

- Add 1.8 mL of distilled water and 40 $\mu$ L of concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) to the Standard powder and dissolve completely to form 40  $\mu\text{mol/mL}$   $\text{FeSO}_4$  standard solution.
- Solution Mixture (Prepare fresh before use): Reagent I: Reagent II: Reagent III=10:1:1, incubate at 37°C for 10 minutes before use.

## Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge,  $\text{H}_2\text{SO}_4$ , ice, spectrophotometer/microplate reader, micro glass cuvette/96-well flat bottom plates and distilled water.

## Sample Preparation

### 1. Serum, Plasma, Saliva or Urine

Centrifuge plasma at 5000 rpm for 10 minutes and use the supernatant for testing. Serum, saliva and urine can be used directly. The samples can be stored at -80°C and used within 30 days.

### 2. Cells

Add 1mL Extraction Solution to 5 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 200W, sonication 3 seconds, interval 7 seconds for 3 minutes). Centrifuge at 10000 rpm at 4°C for 10 minutes. Take the supernatant and place it on ice for assay.

### 3. Tissue

Add 1mL Extract Solution to 0.1g tissue. Use homogenizer or ultrasonicator to fully break up the cells (to be performed on ice). Centrifuge at 10000 rpm for 10 min at 4°C. The supernatant is to be used for the assay.

## Operation Procedures

1. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 593nm and set zero with distilled water.
2. Dilute 40 $\mu\text{mol/mL}$   $\text{FeSO}_4$  standard solution to various concentrations (0.15, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156  $\mu\text{mol/mL}$ ) using distilled water.

- To 100µL of working standard add 100µL Reagent II. Use 100µL distilled water for blank.
- Mix thoroughly for 10 min and measure the absorbance at 593 nm.
- Calculate  $\Delta A = A_S - A_B$ .  
( $A_S$ : Standard solution tube,  $A_B$ : Blank control tube). Final concentration of  $Fe^{2+}$  is 0.075, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156, 0.00078 µmol/mL. to be measured 1-2 times.
- For sample analysis, add the reagents as mentioned.

Reagent	Blank ( $A_B$ )	Test ( $A_T$ )
Solution Mixture (µL)	180	180
Sample (µL)	-	6
Distilled water (µL)	24	18
Mix thoroughly for 10 min, add 200µL to the micro glass cuvette/96-well flat-bottom plates, detect $A_{593}$ calculate $\Delta A' = A_T - A_B$ .		

## Calculations

- Create standard curve  
Plot the final concentration of  $Fe^{2+}$  on the X-axis and  $\Delta A$  on the Y-axis. Create standard curve and obtain linear regression  $y=kx+b$ . Take  $\Delta A'$  into the equation and get x (µmol/mL).
- Unit definition: Antioxidant capacity of the sample is indicated by the standard liquid ion concentration required the same absorbance change ( $\Delta A$ ).

### A. Protein concentration:

$$\begin{aligned}\text{Total antioxidant capacity (µmol/mg prot)} &= x \times V_{rv} \div (V_s \times C_{pr}) \\ &= 34 \times x \div C_{pr}\end{aligned}$$

### B. Sample weight

$$\begin{aligned}\text{Total antioxidant capacity (µmol/g tissue)} &= x \times V_{rv} \div (V_s \div V_{sv} \times W) \\ &= 34 \times x \div W\end{aligned}$$

### C. Cell number

$$\begin{aligned}\text{Total antioxidant capacity (µmol/10}^4\text{cell)} &= x \times V_{rv} \div (V_s \times V_{sv} \div N) \\ &= 34 \times x \div N\end{aligned}$$

### D. Solution volume

$$\begin{aligned}\text{Total antioxidant capacity (U/mL)} &= x \times V_{rv} \div V_s \\ &= 34 \times x\end{aligned}$$

$V_{rv}$ : total reaction volume, 0.204 mL

$V_s$ : sample volume, 0.006 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).

## Notes

- Reagent II is an irritant, please wear lab coat and latex gloves while handling.
- The samples should not appear blue under acidic condition, or it will affect the test result.
- Detergent such as Tween, Triton, NP-40 and reductants such as DTT, mercapto ethanol should not be added in the sample.
- If the absorbance value determined by the sample is beyond the standard curve range, the sample should be diluted or concentrated properly before determination.
- The kit should be store at 2-8°C.