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Ver. 241202

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

BC3305-01 (50 Tests/48 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	50mL	4°C, Precool before use
Reagent I	40mL	4°C
Reagent II	20mL	4°C, Protect from light
Reagent III	5mL	4°C, Protect from light
Standard	20mg ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ )	
	Add 1.8 mL of distilled water and 40µL of concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) to the Standard to form 40 µmol/mL $\text{FeSO}_4$ standard solution.	

### Note:

- Solution mixture: Reagent I: Reagent II: Reagent III=10:1:1, incubate at 37°C for 10 minutes before use. Prepare fresh before use.

## Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer, ultrasonic crusher, ice,  $\text{H}_2\text{SO}_4$ , 1mL glass cuvette 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 or Tissue

Add 1ml Extraction Solution to 5 – 10 million cells or 0.1 – 0.5g tissue. Use homogenizer or ultrasonicator to fully break up the cells (to be performed on ice). Centrifuge at 10000 rpm for 10 minutes at 4°C. The supernatant is to be used for the assay.

## Operation Procedures

1. Preheat the spectrophotometer/microplate reader for 30 min, adjust wavelength to 593 nm and set zero with distilled water.
2. Dilute 40µmol/mL  $\text{FeSO}_4$  standard solution to various concentrations (0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 µmol/mL) using distilled water to get working standard.
3. To 500µL working standards (distilled water as blank), add 500µL Reagent II. Mix

thoroughly and incubate for 10 minutes. Record the absorbance at 593nm.  $\Delta A = A_S - A_B$   
 $A_S$ : Standard solution tube,  $A_B$ : Blank control tube. Final concentration of  $Fe^{2+}$  is 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156  $\mu\text{mol/mL}$

- For sample analysis, add the reagents as mentioned

Reagent	Blank ( $A_B$ )	Test ( $A_T$ )
Solution mixture ( $\mu\text{L}$ )	900	900
Sample ( $\mu\text{L}$ )	-	30
Distilled water ( $\mu\text{L}$ )	120	90
Mix thoroughly and incubate at room temperature for 10 min and 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  $x$  ( $\mu\text{mol/mL}$ ).

- 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:

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

### B. Sample weight

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

### C. Cell number

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

### D. Solution volume

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

$V_{rv}$  : 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).

## Notes

- Reagent II is an irritant, please wear lab coat and latex gloves while handling this.
- 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.