Total antioxidant capacity (T-AOC) Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0768

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 $FeSO_4 \cdot 7H_2O$. Working solution: add 0.9 mL of distilled water and 20 μL of concentrated sulfuric acid (H_2SO_4) to forms 40 μmol/mL $FeSO_4$ 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, Fe^{3+}-TPTZ are reduced to blue 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:

1. Dilute 40 μmol/mL $FeSO_4$ standard solution to 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 μmol/mL, take 500 μL of standard solution (distilled water for blank control), add to 500 μL 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 Fe^{2+} is 0.05、0.025、0.0125、0.00625、0.003125、0.00156 μmol/mL.
2. Preheat the spectrophotometer 30 min, adjust wavelength to 593 nm and set zero with distilled water.
3. Add reagents with the following list:

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

III. Calculation:

1. Create standard curve

   Take the Fe^{2+} final concentration as X-axis, △A as Y-axis, create standard curve, get linear regression equation y=kx+ b, take ΔA' into the equation to get x (μmol/mL).
2. Unit definition: the sample antioxidant capacity is indicated by the standard liquid ion concentration required for the same absorbance change (ΔA).

   A. Protein concentration:

   Total antioxidant capacity (μmol/mg prot) = x × Vrv÷ (Vs× Cpr) = 34× x ÷ Cpr

   B. Sample weight

   Total antioxidant capacity (μmol/g weight) = x × Vrv÷ (Vs ÷ Vsv ×W) =34× x÷ W

   C. Cell amount

   Total antioxidant capacity (μmol/10^4cell) = x × Vrv÷ (Vs ×Vsv÷n) = 34× x÷ n

   D. Solution volume

   Total antioxidant capacity (μmol/mL) =x× Vrv÷ Vs =34×x

   Vrv: total reaction volume, 1.02 mL;
   Vs: sample volume, 0.03 mL;
   Vsv: extraction volume, 1 mL;
   W: sample weight, g;
   Cpr: sample protein concentration, mg/mL;
   n: cell amount, unit based on 10^4 (ten thousand).

Note:

1. 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（μmol/g mass）$=34\times x\div W=34\times 0.037\div 0.1=12.85$ μmol/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:

NA0767/NA0526	Hydroxyl Radical Scavenging Capacity Assay Kit
NA0766/NA0525	Plant Flavonoids Assay Kit
NA0765/NA0524	Plant Total Phenol (TP)Assay Kit