Total antioxidant capacity (T-AOC) Assay Kit (FRAP method)

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer

Catalog Number: BC1310

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution	Solution 60 mL×1	2-8℃
Reagent I	Solution 40 mL×1	2-8℃
Reagent II	Solution 20 mL×1	2-8℃
Reagent III	Solution 6 mL×1	2-8℃
Standard	Powder ×1	2-8℃

Solution preparation:

1. **Extract solution:** Pre cool on 2-8℃ refrigerator or ice.
2. **Standard:** 10 mg of $FeSO_4·7H_2O$. 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.
3. **Mixed solution:** Mix reagent I, reagent II, and reagent III in a ratio of 7:1:1, prepare and use as needed, and mix as much as needed. Preheat for 10 minutes in a 37℃ water bath or a 37℃ constant temperature incubator.

Product Description:

Determine the total antioxidant level composed of various antioxidant substances and antioxidant enzymes in the object. In biological, medical, and pharmaceutical research, the total antioxidant capacity of various body fluids such as plasma, serum, saliva, urine, cell or tissue lysates, plant or herbal extracts, and various antioxidant solutions are often tested.

The ability to reduce Fe^{3+}- triphenyltriazine (Fe^{3+}- TPTZ) to produce blue Fe^{2+}- TPTZ under acidic conditions reflects the total antioxidant capacity.

Technical indicators:

Minimum detection limit: 0.000567243 μmol/mL； Linear range: 0.00078125-0.05 μmol/mL

Reagents and Equipment Required but Not Provided:

Spectrophotometer, 1 mL glass cuvette, water bath/constant temperature incubator, low temperature centrifuge, mortar/homogenizer/cell ultrasonic crusher, sulfuric acid (>95%, AR), ice 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, or they can be frozen at -80℃ (not exceeding 30 days) before measurement.

2. Cells or bacteria samples

   Collect cells or bacteria in centrifuge tubes. According to the ratio of cell or bacterial count (10^4): extract volume (mL) of 500-1000:1, add 1.0mL of pre cooled extract solution (it is recommended to take 5 million cells and add 1mL of pre cooled extract solution), sonicate the cells (power 200W, ultrasound on for 3 seconds, off for 9 seconds, total time for 3 minutes), then centrifuge at 10000rpm, 4℃ for 10 minutes, take the supernatant and place it on ice for testing.

3. Tissue sample

   According to the ratio of tissue mass (g) to extract solution volume (mL) of 1:5-10 (it is recommended to weigh about 0.1g of tissue and add 1mL of pre cooled extract solution), perform ice bath homogenization, then centrifuge at 10000rpm and 4℃ for 10 minutes. Take the supernatant and place it on ice for testing.

II. Determination procedure:

1. Preheat the spectrophotometer for more than 30 min, adjust wavelength to 593 nm and set zero with distilled water.
2. Preparation of standard solution: Dilute 40 μmol/mL standard solution with distilled water to 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 μmol/mL standard solution for later use.
3. The standard solution dilution can refer to the following table:

Number	Pre dilution concentration (μmol/mL)	Standard solution volume (μL)	Distilled water volume (μL)	Post dilution concentration (μmol/mL)
1	40	50	950	2
2	2	100	1900	0.1
3	0.1	1000	1000	0.05
4	0.05	1000	1000	0.025
5	0.025	1000	1000	0.0125
6	0.0125	1000	1000	0.00625
7	0.00625	1000	1000	0.003125

Note: Each standard tube in the following experiment requires 500 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

4. 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. The standard curve only needs to be done 1-2 times.
5. Operation table:

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 accurately at room temperature for 10 minutes. Take 1mL and measure the absorbance at 593nm in a 1mL glass cuvette. Calculate $\Delta A_T=A_T - A_B$. The blank tube only needs to be measured 1-2 times.		

III. Calculation:

1. Create standard curve

   Establish a standard curve based on the final concentration of Fe^{2+} (x, μmol/mL) and absorbance ΔA standard (y, ΔA_S). According to the standard curve, calculate the sample concentration (x, μmol/mL) by substituting the ΔA_T (y, ΔA_T) into the formula.

2. Formula

   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 ÷ Ve ×W) =34× x÷ W

   C. Cell amount

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

   D. Solution volume

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

   Ve: extract solution volume, 1 mL;

   Vrv: total reaction volume, 1.02 mL;

   Vs: sample volume, 0.03 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 irritating to the human body, please take appropriate protective measures. For your safety and health, please wear lab coats and latex gloves when operating.

   Try to avoid using samples that appear blue or close to blue under acidic conditions, otherwise it may interfere with the detection results of this kit.

2. It is not advisable to add descaling agents such as Tween, Triton, and NP-40, as well as reducing agents such as DTT

and mercaptoethanol that affect redox reactions, to the sample.

3. If the absorbance value exceeds the linear range, the sample size can be increased or the sample can be diluted before proceeding with the measurement. Pay attention to synchronously modifying the calculation formula.

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: ΔA $=A_T-A_B=0.909-0.148=0.761$, standard curve: y=21.056x-0.0087, calculate x=0.037, according with mass of sample to calculate:

   Total antioxidant capacity (μmol/g weight) =34×x÷W=34×0.037÷0.1=12.58 μmol/g weight.

Recent Product citations:

[1] Liu S, Meng F, Zhang D, Shi D, Zhou J, Guo S, Chang X. Lonicera caerulea Berry Polyphenols Extract Alleviates Exercise Fatigue in Mice by Reducing Oxidative Stress, Inflammation, Skeletal Muscle Cell Apoptosis, and by Increasing Cell Proliferation. Front Nutr. 2022 Mar 9;9:853225. doi: 10.3389/fnut.2022.853225. PMID: 35356725; PMCID: PMC8959458.

[2] Geng X, Xue R, Liang F, Liu Y, Wang Y, Li J, Huang Z. Synergistic effect of silver nanoclusters and graphene oxide on visible light-driven oxidase-like activity: Construction of a sustainable nanozyme for total antioxidant capacity detection. Talanta. 2023 Jul 1;259:124565. doi: 10.1016/j.talanta.2023.124565. Epub 2023 Apr 17. PMID: 37084604.

[3] Liu S, Yu J, Fu M, Wang X, Chang X. Regulatory effects of hawthorn polyphenols on hyperglycemic, inflammatory, insulin resistance responses, and alleviation of aortic injury in type 2 diabetic rats. Food Res Int. 2021 Apr;142:110239. doi: 10.1016/j.foodres.2021.110239. Epub 2021 Feb 25. PMID: 33773689.

[4] Liu S, Jiang W, Liu C, Guo S, Wang H, Chang X. Chinese chestnut shell polyphenol extract regulates the JAK2/STAT3 pathway to alleviate high-fat diet-induced, leptin-resistant obesity in mice. Food Funct. 2023 May 22;14(10):4807-4823. doi: 10.1039/d3fo00604b. PMID: 37128963.

[5] Zhu X, Guo R, Su X, Shang K, Tan C, Ma J, Zhang Y, Lin D, Ma Y, Zhou M, Yang J, Wu Q, Sun J, Wang Z, Guo Y, Su R, Cui X, Han J, Lü Y, Yue C. Immune-enhancing activity of polysaccharides and flavonoids derived from Phellinus igniarius YASH1. Front Pharmacol. 2023 Apr 25;14:1124607. doi: 10.3389/fphar.2023.1124607. PMID: 37180713; PMCID: PMC10166811.

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:

BC1320/BC1325 Hydroxyl Radical Scavenging Capacity Assay Kit

BC1330/BC1335 Plant Flavonoids Assay Kit

BC1340/BC1345 Plant Total Phenol (TP) Assay Kit

BC1350/BC1355 Plant Proanthocyanidins Assay Kit

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