Total Antioxidant Capacity (T-AOC) Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC1315

Size: 100T/96S

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 100 mL×1	2-8℃
Reagent I	Solution 15 mL×1	2-8℃
Reagent II	Solution 6 mL×1	2-8℃
Reagent III	Solution 2 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/microplate reader, micro glass cuvette/96-well plate, water bath/constant temperature incubator, low temperature centrifuge, mortar/homogenizer/cell ultrasonic crusher, sulfuric acid (>95%, AR), ice and distilled water.

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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/microplate reader 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 0.15, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156 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	75	925	0.15
3	2	50	950	0.1
4	0.1	200	200	0.05
5	0.05	200	200	0.025
6	0.025	200	200	0.0125
7	0.0125	200	200	0.00625
8	0.00625	200	200	0.003125
9	0.003125	200	200	0.00156

Note: Each standard tube in the following experiment requires 100 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

4. Take 100 μL of standard solution (distilled water for blank control), add to 100 μL of Reagent II.

Mix

thoroughly for 10 min and detect the absorbance in 593nm, calculate $\Delta A=A_S-A_B$. (A_S: standard solution tube, A_B: blank control tube.) The final concentration of Fe^{2+} is 0.075、0.05、0.025、0.0125、0.00625、0.003125、0.00156、0.00078 μmol/mL. The standard curve only need to be measured 1-2 times.

5. Operation table

Reagent Name	Blank Tube (A_B)	Test Tube (A_T)
Solution Mixture(μL)	180	180
Sample (μL)	-	6
Distilled Water(μL)	24	18
Mix thoroughly and react for 10 min, add 200 μL to the micro glass cuvette/96-well flat-bottom plates, detect A_{593} 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

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

Vrv: total reaction volume,0.204 mL;

Vs: sample volume, 0.006 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 irritating to the human body, please take appropriate protective measures. For your safety and health, please wear lab coats and latex gloves when operating.
2. 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.
3. 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.
4. 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 plate to calculate: $\Delta A=A_T - A_B=0.490-0.139=0.351$, standard curve: y=14.039x-0.0029, calculate x=0.025, according with mass of sample to calculate:

Total antioxidant capacity（μmol/g weight）=34×x÷W=34×0.025÷0.1=8.5 μmol/g weight.

Recent Product Citations:

[1] Yu Y, Hu L, Tian D, Yu Y, Lu L, Zhang J, Huang X, Yan M, Chen L, Wu Z, Shi W, Liu G. Toxicities of polystyrene microplastics (MPs) and hexabromocyclododecane (HBCD), alone or in combination, to the hepatopancreas of the whiteleg shrimp, Litopenaeus vannamei. Environ Pollut. 2023 Jul 15;329:121646. doi: 10.1016/j.envpol.2023.121646. Epub 2023 Apr 25. PMID: 37105466.

[2] He F, Shi H, Liu R, Tian G, Qi Y, Wang T. Randomly-shaped nanoplastics induced stronger biotoxicity targeted to earthworm Eisenia fetida species: Differential effects and the underlying mechanisms of realistic and commercial polystyrene nanoplastics. Sci Total Environ. 2023 Jun 15;877:162854. doi: 10.1016/j.scitotenv.2023.162854. Epub 2023 Mar 16. PMID: 36931517.

[3] Bao Z, Zhu Y, Feng Y, Zhang K, Zhang M, Wang Z, Yu L. Enhancement of lipid accumulation and docosahexaenoic acid synthesis in Schizochytrium sp. H016 by exogenous supplementation of sesamol. Bioresour Technol. 2022 Feb;345:126527. doi: 10.1016/j.biortech.2021.126527. Epub 2021 Dec 9. PMID: 34896539.

[4] Bao Z, Zhu Y, Zhang K, Feng Y, Chen X, Lei M, Yu L. High-value utilization of the waste hydrolysate of Dioscorea zingiberensis for docosahexaenoic acid production in Schizochytrium sp. Bioresour Technol. 2021 Sep;336:125305. doi: 10.1016/j.biortech.2021.125305. Epub 2021 May 21. PMID: 34044242.

[5] Du Y, Zhang S, Sun-Waterhouse D, Zhou T, Xu F, Waterhouse GIN, Wu P. Physicochemical, structural and emulsifying properties of RG-I enriched pectin extracted from unfermented or fermented cherry pomace. Food Chem. 2023 Mar 30;405(Pt B):134985. doi: 10.1016/j.foodchem.2022.134985. Epub 2022 Nov 17. PMID: 36442238.

References:

[1] Madhubalaji C, Mudaliar S, Chauhan V. et al. Evaluation of drying methods on nutritional constituents and antioxidant activities of Chlorella vulgaris cultivated in an outdoor open raceway pond[J]. Journal of Applied Phycology, 2021, 33: 383-399.

[2] Quan Huali, Li Wenhui, Liang Xinmei. et al. Effect of pre-defatting heat treatment on active substances and in vitro antioxidant capacity of sesame meal[J]. Food and Fermentation Industries, 2021, 13: 133-139.

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 (OPC) Assay Kit

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