SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

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

Cat No: AK0455

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Extract Solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Liquid 6 mL×1	2-8℃
Reagent III	Liquid 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 $FeSO_4 \cdot 7H_2O$. 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: According to the sample size, mix Reagent I, Reagent II, and Reagent III=700μL:100μL: 100μL(900μL,5T). Prepare it for immediate use.

Product Description:

Determine the total antioxidant level composed of various antioxidant substances and antioxidant enzymes in the sample. 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: 0.00078125-0.1 μmol/mL

SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Reagents and Equipment Required but Not Provided:

Visible 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.

Operation procedure

I. Sample Preparation:(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 testing. 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 solution (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. The mixed solution should be equilibrated at room temperature for 10 minutes before use.

6. Operation table(Add the following reagents in sequence into a 96 well plate or EP tube)

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 at room temperature, add 200 μL to the micro glass cuvette/96-well flat-bottom plates.Measure the absorbance value at 593nm and calculate $\Delta A'=A_T-A_B$. (Note: The blank tube just need to be tested once or twice in every experiment.)		

SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

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 mass

Total antioxidant capacity (μmol/g mass) = 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: Extract solution volume, 1 mL;

W: sample mass, 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.

Experimental example:

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 mass)=34×x÷W=34×0.025÷0.1=8.5 μmol/g mass.

Related Products:

AK0454/AK0453 Hydroxyl Radical Scavenging Capacity Assay Kit
AK0452/AK0451 Plant Flavonoids Assay Kit
AK0450/AK0449 Plant Total Phenol (TP)Assay Kit
AK0448/AK0447 Plant Proanthocyanidins Assay Kit