Total Antioxidant Capacity (T-AOC) Assay Kit

Cat No. TAC-M-001-50
Size: 50 Reactions
Store at 4°C

MOLEQULE-ON®

Components	Quantity
Extraction Reagent	50ml
Reagent I	35ml
Reagent II	20ml
Reagent III	5ml
Standard: $FeSO_4 \cdot 7H_2O$	10mg

Description

Total antioxidant capacity is made up primarily for antioxidants and antioxidant enzymes. It can detect total antioxidant capacity of all kinds of antioxidant like serum, plasma, cell or tissue lysate and others.
The total antioxidant capacity can be reacted by reducting Fe^{3+} - three pyridine three azine (Fe^{3+} -tptz) to blue Fe^{2+}-tptz in acidic environment.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, low temperature centrifuge, cuvette, distilled water.

Preparations

Standard Preparation: In 10mg of $FeSO_4 \cdot 7H_2O$ add 1.75ml distilled water and 1 drop of concentrated sulfuric acid to form 20μmol/mL $FeSO_4$ standard solution.
Mixture: Reagent I: Reagent Ⅱ: Reagent Ⅲ = 7:1:1, keep at 37℃ before using.

Protocol

I. Sample preparation:

1. Serum, plasma, saliva or urine samples
Plasma should be taken in sodium citrate vacutainer or alternatively in heparin. Centrifuge at 5000 rpm for 10min, take supernatant for test. Detect directly or after cryopreserving in -80℃ (within 30 days).

2. Cell or tissue sample
Take 100-200 million cells or 0.1g tissue, adding 1.0ml pre-cooled extraction reagent, homogenize cells by vortexing or fully broken cells with ultrasonic bath, centrifuge at 10,000 rpm for 5min at 4℃, take supernatant for test.

II. Determination procedure:

1. Mark 8 tubes with 0.2, 0.1, 0.05, 0.0125, 0.00625, 0.003125, 0.00156 μmol/mL and blank. Take 10μL from 20μmol/mL $FeSO_4$ standard solution and add in 990μL of distilled water in the tube marked as 0.2. Take 500μL from 0.2μmol/mL standard and pipette out in 0.05μmol/mL tube, likewise take 500μL from 0.05 tube and pour in 0.0125 standard. Serially dispense 500μL from each standard to the next standard marked above. Add 500μL Reagent Ⅱ in each standard tube and mix thoroughly for 10 min, set zero in spectrophotometer with double distilled water and take reading at 593nm, calculate ΔA=A(S)-A(B), the final concentration of Fe^{2+} is 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156, 0.00078 μmol/mL.

2. Preheat the spectrophotometer for 30 minutes, adjust wavelength to 593 nm, set zero with distilled water.

3.

	Contrast tube (C)	Test tube (T)
Mixture (μL)	900μL	900μL
Sample (μL)		30μL
Double distilled water (μL)	120μL	90μL
Mix thoroughly for 10min, set zero with distilled water, 1cm light path, detect A_{593} calculate ΔA'=A(T)-A(C).		

Calculations

1. **Draw standard curve**
Take the Fe^{2+} final concentration as X-axis, △A as Y-axis, draw standard curve, get y=kx+b and x according to △A.
2. **Unit definition**: the antioxidant capacity of sample showed by the needed ionic concentration of standard solution to the same absorbance △A.

A. Protein concentration:

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

B. Sample weight

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

C. Cell

Total antioxidant capacity (U/10^4cell) = x × Vrv÷ Vs ×Vsv÷N (10^4) or
= 34×x÷N (10^4)

D. Solution volume

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

Vrv: reaction total volume, 1.02 mL;
Vs: the sample volume,0.03 ml;
Vsv: the extraction volume, 1 ml;
W: sample weight, g;
Cpr: sample protein concentration, mg/mL;
N: cell quantity, (10^4).

Notes:
1. Wear latex gloves and clothing for the safe.
2. Do not use blue or nearly blue sample to avoid interfering.
3. Do not add detergents like Tween, Triton, NP-40 and DTT to avoid influencing reaction.
4. Dilute or concentrate sample if the absorbance outside of standard curve.