For research use only.

Total antioxidant capacity(T-AOC) Assay Kit

(abk100 FRAP method 96T)

Reagents composition & Preparation

Reagents	Composition	Position	Storage
R1	Check buffer	15ml×1 bottle	Store at -20℃ for 1 year
R2	Matrix solution	1.5ml×1 vial	Store at -20℃ and preserve avoid the light for 1 year
R3	Substrate solution	1.5ml×1 vial	Store at -20℃ and preserve avoid the light for 1 year
FRAP working solution configuration: In accordance with reagent I: Reagent II: Reagent III 10:1:1 well mixed,incubate at 37 °c and avoid the light ,prepare when you use and used up within 2 hours.			
R4	$FeSO_4$-$7H_2O$ Standard	100mg	Store at -20℃ for 1 year
It comes with a disposable 96-hole plate			

Intended use

The kit is used for the determination of total antioxidant capacity in serum, plasma, tissue homogenate, cells (or supernatants) , saliva and urine.

Assay principle

Under acidic conditions, the antioxidant can reduce Fe^{3+}-TPTZZ to produce blue Fe^{2+}-TPTZ. The total antioxidant capacity can be calculated by reading the absorbance at 593 nm

Determinate significance

There are many kinds of antioxidant macromolecules, small molecules and enzymes in the body, which can eliminate all kinds of reactive oxygen species produced in the body, thus preventing the generation of oxidative stress induced by reactive oxygen species.The total level of various antioxidant macromolecules, small antioxidant molecules and enzymes in a system reflects the total antioxidant capacity of the system.Therefore,

it is of great significance to determine the total antioxidant capacity in serum and other body fluids, tissues and cell lysate.

Applicable equipment： Various types of photometer or microsamples can be determined.

Sample preparation

1、Preparation of Serum, Saliva, urine, supernatant and other liquid samples：

Blood samples should be separated to get serum or plasma in time after collection. hemolysis should be avoided during sampling. Direct determination of saliva, urine and supernatant of cells. **Plasma is recommended for Heparin or Sodium Citrate anticoagulation and should not be treated with EDTA**.Literature report,The T-AOC of human serum was 0.5 ～ 2mM;The T-AOC of human saliva was 0.3-1mM

2、Animals（Plants）Tissue：

The weight of the tissue was measured accurately, and the ratio of weight (g) : Volume (ml) 1:4, adding 4 times volume of normal saline or PBS, mechanical homogenization under ice-water bath condition, fully crushing the cells to release the antioxidants,centrifuged at 4℃,12000rpm for 5 minutes, and take the supernatant to determination.

3、Cell:

The collection of not less than 1 million cells is recommended for cell scraping and should not be digested with trypsin and EDTA. Add 200μLcold PBS, homogenate or ultrasound to fully crush the cells and release their antioxidants,centrifuged at 4℃,12000rpm for 5 minutes, and take the supernatant to determination

Operation Procedure

	Blank	**Standard**	**Sample**
Distilled water（μl）	5		
Different concentrations of Standard solution（μl）		5	
Sample （μl）			5
FRAP working solution（μl）	180	180	180
The OD values of each hole were determined by enzyme-labeled method at 593 nm (or within 585-605 nm) after 3-5min reaction at 37℃			

Standard solution configuration of different concentrations：

The concentration of $FeSO_4$-$7H_2O$ is 100mM when the solution of 27.8mg $FeSO_4$-$7H_2O$ is dissolved and the volume reaches 1ml. Appropriate amount of 100mM $FeSO_4$-$7H_2O$ solution was diluted to 0.15,0.3,0.6,0.9,1.2 and 1.5 mm. It is usually possible to dilute the reference material with a solution of distilled water or sample preparation.

Note 1: For liquid samples such as serum, Plasma, saliva or urine, **distilled wate**r or **PBS** is recommended for the preparation of the reference substance; for cell or tissue samples, a **homogenate medium** for homogenization of cells or tissues is recommended for preparation of the reference substance, other samples refer to the preceding method.

Note 2： $FeSO_4$ solution should be prepared and used fresh. 100mM $FeSO_4$ solution is easy to oxidize to produce ferric salt, which changes the color of the solution from light green to light yellow. If it is found that the colour of the solution is obviously yellow, the solution should be discarded and the fresh FESO4 solution should be reformulated using the $FeSO_4$-$7H_2O$ provided by the kit.

Calculate

Take the standard OD value as the horizontal coordinate and the standard OD concentration as the vertical coordinate to make the Standard Curve.Get the curve formula by drawing software (or excel table),Take the OD value measured by the sample into the calculation formula , and then obtain the results.

Expression of T-AOC

The total antioxidant capacity is expressed by the concentration of $FeSO_4$ standard solution.For example, if the OD value of a serum sample is the same as that of 1mM FESO4, then the total antioxidant capacity of the serum sample is 1mM;For example, the OD value of tissue homogenate or cell homogenate was the same as that of 3mM $FeSO_4$. The total antioxidant capacity of tissue homogenate or cell homogenate was 3mM/(1mg/ml) ,that is 3mmol/gprot, when the protein concentration of tissue homogenate or cell homogenate was 1mgprot/ml.

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Points for attention：

1 、 Reagents that are blue or near-blue in acidic conditions may interfere with the results and should be avoided.

2、If the sample contains high concentration of iron salt or ferrous salt, will interfere with the determination, because in acidic conditions can inhibit the interference of endogenous substances in the sample. If the total concentration of iron or ferrous ions in the serum of a sample is always below 10 m, it will not interfere with the FRAP method. The presence of a small amount of the metal chelating agent in the sample does not affect the determination.

3、 It is not advisable to add DTT, Beta-mercaptoethanol and other substances that affect Redox, nor to add scale removers such as Tween, Triton and NP-40.

4、If the sample can not be detected in time, it is recommended-80℃ frozen storage, within a month of the measured data did not change significantly

5、This kit is for scientific research only, not for clinical diagnosis or treatment, not for food and medicine.

6、Reagent 2 is irritating to human body, please wear lab coat and operate with gloves.