Total Antioxidant Capacity Assay Kit (FRAP)

Catalog Number: K025

Storage Temperature:-20℃ is valid in 12 months

Product Description

Oxidants, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), can generate free radicals that can cause severe oxidative damage to cellular lipids, membranes, proteins, and DNA. Antioxidants can scavenge these free radicals and prevent cellular oxidative stress by enzymatic and non-enzymatic mechanisms. Enzyme systems that function as antioxidants include catalase and peroxidase. Tocopherols, carotenes, vitamin A, and ubiquinols function as lipid-soluble antioxidants; whereas, glutathione and ascorbate are some of the water-soluble antioxidants. Measurement of the total non-enzymatic antioxidant capacity (TAC) of biological samples is indicative of their ability to counteract oxidative stress-induced damage in cells. TAC is used to provide insights into the development and treatment of oxidative-stress related disorders.

In the Total Antioxidant Capacity Assay Kit, either the concentration of the combination of both small molecule and protein antioxidants, or the concentration of only small molecule antioxidants can be determined. Under acidic condition, Fe^{3+}- TPTZ is converted to Fe^{2+}- TPTZ by both small molecules and proteins. The reduced Fe^{2}+ion chelates with a colorimetric probe, giving a broad absorbance peak at 593 nm, which is proportional to the total antioxidant capacity. Because of acidic conditions, and the total plasma concentration of iron ion and ferrous ion in serum samples is usually lower than 10 μM, some endogenous interference factors can be inhibited.

Antioxidant

Fe^{3+}-TPTZ ————————> Fe^{2+}-TPTZ (blue)

The kit gives antioxidant capacity in Trolox equivalents. Trolox, a water-soluble vitamin E analog, serves as an antioxidant standard.

Components

The kit is sufficient for 100 assays in 96 well plates.

TPTZ Diluent	15 mL
TPTZ solution	1.5 mL
Detection buffer	1.5 mL
$FeSO_4 \cdot 7H_2O$	200mg
Trolox Standard, 10mM	0.1 mL

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Preparation of FRAP working solution:

Refer to the following table, according to the number of samples to be measured (including standard curve) to prepare the right amount of FRAP working solution:

Number of samples	1	5	10	20	50
TPTZ Diluent	150μl	750μl	1500μl	3000μl	7500μl
TPTZ solution	15μl	75μl	150μl	300μl	750μl
Mix well and then add the buffer					
Detection buffer	15μl	75μl	150μl	300μl	750μl
FRAP working solution	180μl	900μl	1800μl	3600μl	9000μl

FRAP working solution should be incubated at 37℃, and used within 1-2 hours.

Storage/Stability

The kit is shipped on ice pack and storage at –20 ℃for 12 months. TPTZ solution, Detection buffer, Trolox Standard is recommended to protect from light.

Procedure

All samples and standards should be run in duplicate.

Standards for Colorimetric Detection

Take 27.8 mg $FeSO_4 \bullet 7H_2O$ provided by this kit, dissolve and hold to a concentration of 1 ml, at this time the concentration of $FeSO_4 \bullet 7H_2O$ is 100 mM. Then dilute100 mM $FeSO_4 \bullet 7H_2O$ to 0.15, 0.3, 0.6, 0.9, 1.2 and 1.5mM with PBS.

The $FeSO_4$ solution should be freshly prepared. 100mM $FeSO_4$ solution is easy to be oxidized to ferric, making the color from initial light green gradually to light yellow. If the color is yellow, abandon it and make fresh solution.

Sample Preparation

1. Serum, plasma, saliva, urine, and culture media samples can be directly added to the wells.

For plasma samples, it is suggested to use heparin or sodium citrate, not EDTA, for anticoagulation. According to the literature, the total antioxidant capacity of human serum or plasma was 0.5-2mM, saliva was 0.3-1mM, urine was 0.2-3mM.

2. For cells or tissue samples, supernatant of prepared cells or tissue samples can be directly added to the wells. At the same time, protein concentration should be tested; final determination of total antioxidant capacity was usually expressed as the total antioxidant capacity per gram or microgram of protein, indicating a unit of mmol/mg or mmol/g.

3. For other samples, Plant or Chinese herbal extractcan be directly added to the wells, total antioxidant capacity can be expressed mmol/mg or mmol/g. If the concentration of antioxidant can be expressed as molar concentration, the total antioxidant capacity measured can be expressed by the relative total antioxidant capacity. For example, the measured absorbance of a 0.5mM antioxidant is the same as that of 1mM $FeSO_4$, the relative total antioxidant capacity was 2.

Assay Reaction

1. Add 180μL of FABP Working Solution to all standard and sample wells.

2. Aliquot 5μl of0.15, 0.3, 0.6, 0.9, 1.2 and 1.5mM standard solutions into the standard wells.

3. Add 5μl of ddH2O or PBS into the control (zero) well.

4. Add 5μl of 0.15-1.5mMTrolox into a sample well as positive control; add 5μl of each sample into other sample wells.

5.Seal the plate with a cover and incubate at 37 °C for 3-5min. Measure the absorbance at 593 nm (A593).If A593 is difficult to measure, it can be measured in the range of 585-605nm.

6. The total antioxidant capacity of the sample was calculated according to the standard curve. If the absorbance of the sample was beyond the standard curve, the sample should be diluted and then determined.

7. For FRAP method, the total antioxidant capacity is expressed with concentration of $FeSO_4$ standard solution. For example, if the measured absorbance of a plasma or serum sample is the same with that of1mM $FeSO_4$, the total antioxidant capacity of the plasma or serum sample is 1mM; if the measured absorbance of a cell homogenate is the same with that of0.3mM $FeSO_4$, and its protein concentrations 0.15mg/ml, the total antioxidant capacity of the cell homogenate sample is 0.3mM/0.15mg/ml, namely 2mmol/g; if the measured absorbance of a 0.2mM antioxidant is the same as that of 1mM $FeSO_4$, the relative total antioxidant capacity was 5.