Total Antioxidant Capacity Assay Kit (FRAP)

Catalog No.: K025

Size:100T

Storage: -20℃ for 12 months.

Kit components:

Reagents	Quantity	Storage
TPTZ Diluent	15ml	-20°C
TPTZ solution	1.5ml	-20°C, avoiding light
Detection buffer	1.5ml	-20°C, avoiding light
FeSO4·7H2O	1.5g	-20°C, avoiding light
Positive Control (2mM)	2ml	-20°C

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, Fe3+-TPTZ is converted to Fe2+-TPTZ by both small molecules and proteins. The reduced Fe2+ion chelates with a colorimetric probe, giving a broad absorbance peak at 593 nm, which is proportional to the total antioxidant capacity. Because the reaction is carried out under acidic conditions, some endogenous interfering factors can be suppressed.And because the total concentration of iron or ferrous ions in samples such as plasma is usually less than 10μM, the iron or ferrous ions in samples such as plasma do not significantly

interfere with the detection response of FRAP.Since iron or ferrous ions in the reaction system are chelated with TPTZ, the small amount of metal ion chelating agent contained in the sample itself usually does not significantly affect the detection reaction.

Antioxidant

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

Precautions:

- Blue or close to blue reagents can interfere with the detection of this kit and should be avoided as much as possible. If the sample contains a higher concentration of iron salt, the determination will be disturbed. However, trace amounts of iron salts in samples such as plasma, serum, cell or tissue lysate do not interfere with the assay.
- Don't add DTT, BME, Tween or Triton to the sample .
- The determination needs a spectrograph that can measure A593(585-605nm can also).
- TPTZ is irritating to the human body, please be careful when operating, avioding direct contact or inhalation.
- This product is limited to research, shall not be used for clinical diagnosis or treatment, shall not be used in food or medicine, and shall not be stored in ordinary residences. For your safety and health, wear lab clothes and disposable gloves when operating.

Sample Preparation:

- Serum, plasma, saliva or urine samples require 20 μl each can be used directly for assays, or can be frozen at -80°C before measuring. There was no significant change in at least one month. Heparin or sodium citrate can be used for plasma preparation and EDTA should not be used.

According to literature, the total antioxidant capacity in human serum or plasma is 0.5-2 mM, 0.3- 1 mM in human saliva and 0.2-3 mM in urine.

- For cell samples, collecting about 1 million cells (without accurate counting, direct scraping, not suitable for digestion using pancrease and EDTA), placed in 200 ml of cold PBS, homogenizing or ultrasound to fully break the cells and release the antioxidants in them, 12,000g centrifugate at 4°C for 5 minutes, collect supernatant for assay.
- For tissue samples, add 100 microliters of cold PBS to each 20 mg tissue, homogenize or ultrasound to fully break the tissue and release the antioxidants in it, 12,000g centrifugate at 4° C for 5 minutes, collect supernatant for assay. All of the above operations need to be performed on ice. If the preparation of cell or tissue samples is not immediately used for assay, it can be frozen at -80°C. There was no significant change in at least one month.

Standard curve preparation:

- The $FeSO_4$ solution should be freshly prepared for it is easy to be oxidized to ferric. If the color turn from light green to light yellow, discard it and prepare a new one .
- Dissolve 13.9 mg $FeSO_4 \cdot 7H2O$ in 1 ml PBS, dilute the solution 10 times to make the concentration of 5 mM.
- Prepare standard curve dilution as described in the table in 96 well plates.

Concentration (mM)	$FeSO_4$ solution (µl)	dH_2O (µl)
1.5	6	14
1	4	16
0.75	3	17
0.5	2	18
0.25	1	19
0	0	20

Assay solution preparation:

- Mix the TPTZ Diluent, TPTZ solution, and Detection buffer with ratio of 10: 1 : 1 to make the assay solution. Prepared for immediate use, operated on ice .
- Add 20 µl sample, positive control and deionized wate (blank control) to the wells.
- Add 180µl of assay solution to each well.
- Measure A593 (585-605nm can also) after incubation at 37°C for 3-5 minutes.

Data Analysis:

- Calculate the total antioxidant capacity of the sample according to the standard curve (curve expert recommended) .
- If the measured absorbance of the sample is outside the range of the standard curve, the sample needs to be appropriately diluted before the measurement.

Typical Data: