A015-2-1 96 Tests

Total Antioxidant Capacity Assay Kit

Organism Species: *Pan-species* (General)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition

[INTENDED USE]

The kit is a ABTS method for the in vitro quantitative measurement of total antioxidant capacity within serum, plasma, tissue homogenate, cells (or cell culture supernates).

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity(96T)	Reagents	Quantity(96T)
Reagent 1	1×20ml	Reagent 4	1×0.2ml
Reagent 2	1×1ml	Reagent 5	1×0.1ml
Reagent 3	1×0.5ml	96-well strip plate	1
Instruction manual	1/1		

[MATERIALS REQUIRED BUT NOT SUPPLIED]

Plate readers with light filters of desired wavelength (405-425nm)

[STORAGE OF THE KITS]

1. Reagent 1: Buffer Solution. Can be stored at -20℃ for 12 months.
2. Reagent 2: ABTS Solution. Can be stored at -20℃ for 12 months. Avoid Illumination.
3. Reagent 3: H_2O_2 Stock Solution. Can be stored at -20℃ for 12 months.
4. Reagent 4: Peroxidase Stock Solution. Can be stored at -20℃ for 12 months.
5. Reagent 5: 10mM Trolox Solution. Can be stored at -20℃ for 12 months. Avoid Illumination.

[REAGENT PREPARATION]

1. **Reagent 3 Solution Preparation:** Dilute the stock solution with double distilled water (DDW) to 40 times of its original volume.
2. **ABTS Solution Preparation:** Blend Reagent 1, Reagent 2 and Reagent 3 solution with the ratio of 76:5:4 in order to prepare the desired amount of ABTS solution. ABTS Solution should be preserved at RT without light and used within 30 min.
3. **Reagent 4 Solution Preparation:** Dilute the given reagent 4 with reagent 1 to the 10 times of its original volume. Reagent 4 solution should be prepared right before its usage with the amount needed.

[SAMPLE PREPARATION]

1. Aqueous Sample like Serum/Plasma

Pretreatment: Measure directly. Note, for plasma samples, EDTA is not a recommended coagulant.

2. Tissue Sample

Pretreatment: Weight the sample precisely and add the saline with ratio of 1g sample with 9ml saline. Homogenize the mixture in the ice water bath and the centrifuge the homogenate at 4℃, 12000 rpm for 5min. Extract the supernatant for further measurement.

3. Cells

Pretreatment: Collect no less than 1 million cells and add 200μl cold phosphate buffer solution. Disrupt the cells either by homogenization or sonication, and then centrifuge the homogenate at 4℃, 12000 rpm for 5min. Extract the supernatant for further measurement.

Note: For tissue sample and cells, calculation requires the protein concentration of the homogenate. It is recommended to use IS003 BCA Protein Quantification Kit to determine the protein concentration.

[ASSAY PROCEDURE]

Operation table:

	Blank	Standard	Sample
Distilled Water (ul)	10		
Trolox Solution (ul)		10	
Sample (ul)			10
Reagent 4 Solution (ul)	20	20	20
ABTS Solution (ul)	170	170	170
React at RT for 6 min. Read the optical density (OD) at 414nm with a plate reader.			

Note: The 10 mM Trolox standard solution was diluted to 0.1 mM, 0.2 mM, 0.4 mM, 0.8 mM and 1.0 mM by double evaporation.

[TEST PRINCIPLE]

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) can be oxidized to greenish $ABTS^{+}$ in the presence of proper oxidants. The production of $ABTS^{+}$ can be inhibited with antioxidants and thus the total antioxidant capacity can be calculated based on the optical density of $ABTS^{+}$ at 414 or 734nm. Trolox is a water-soluble analog of vitamin E with the similar anti-oxidative capability and is applied as the antioxidant capacity equivalency.

$$ABTS^{\cdot+} \xrightarrow{\text{Antioxidant}} > ABTS$$

[CALCULATION OF RESULTS]

The total antioxidant capacity of the sample is calculated based on the standard curve made. From the OD values of the sample, the Trolox equivalent antioxidant capacity of the pretreated sample can be obtained.

For tissue sample and cells

$$\frac{T-AOC}{mM/mgprot} = \frac{T-AOC}{\mathrm{m}M/ml}(\mathrm{Pretreated}) \div \frac{C_{pr}}{mgprot/ml}$$