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Datasheet

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Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit

Cat# CC1051 – 50/100 Assays

Storage at 4℃ for 6 months

APPLICATION

The kit is used for the determination of total antioxidant capacity (T-AOC) in serum, plasma, saliva, urine, tissue, cells samples.

DETECTION SIGNIFICANCE

There are two kinds of antioxidant system, one is enzyme antioxidant system, including superoxide dismutase (SOD),) catalase (CAT), glutathione peroxidase (GSH-Px). The other is non-enzymatic antioxidant systems, including uric acid, vitamin C, vitamin E, glutathione, bilirubin, α-lipoic acid, carotenoid. Antioxidant capacity is thought to be the cumulative effect of all antioxidants in blood and body fluids.

DETECTION PRINCIPLE

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflect the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm.

- **Method:** Colorimetric method
- **Specification:** 50/100 Assays
- **Measuring instrument:** Spectrophotometer
- **Sensitivity:** 0.62 U/mL
- **Detection range:** 0.62-145.2 U/mL

KIT COMPONENTS

Item	Component	Specification, 50 assays	Specification, 100 assays	Storage
Reagent 1	Buffer Solution	60 mL x 1 vial	60 mL x 2 vials	2-8℃, 6 months
Reagent 2	Chromogenic Agent	Powder x 1 vial	Powder x 2 vials	2-8℃, 6 months, shading light
Preparation of Reagent 2 working solution: Dissolve a vial of powder with 120 mL double-distilled water fully (It can be dissolved by incubating in 80-90℃ water bath). It can be used after cooling to room temperature.				
Reagent 3	Ferric Salt Stock Solution	1.5 ml × 1 vial	1.5 ml × 2 vials	2-8℃, 6 months, shading light
Reagent 4	Ferric Salt Diluent	30 mL x 1 vial	60 mL x 1 vial	2-8℃, 6 months
Preparation of Reagent 3 working solution: Dilute the reagent 3 with reagent 4 at the ratio of 1:19. Prepared the fresh solution before use.				
Reagent 5	Stop Solution	12 mL x 1 vial	24 mL x 1 vial	2-8℃, 6 months
Reagent 6	Clarificant	12 mL x 1 vial	24 mL x 1 vial	

EXPERIMENTAL INSTRUMENT

Micropipettor, Vortex mixer, Thermostat incubator, Centrifuge, Spectrophotometry (520 nm), Balance.

PRETREATMENT OF SAMPL

Sample requirements: The sample should not contain DTT, 2-mercaptoethanol and other reducing agents.

1. **Serum sample:**

   Fresh blood was collected and placed at 25℃ for 30 min to clot the blood. Centrifuge the sample at 4 ℃ for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection. If not detected on the same day, stored the serum at-80℃, which can be stored for a month.

2. **Plasma sample:**

   The fresh blood was added into the test tube containing anticoagulant and mixed upside down. Centrifuge the sample at 4℃ for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection. If not detected on the same day, stored the serum at-80℃, which can be stored for a month.

3. **Tissue samples:**

   Take 0.02-1 g tissue sample, wash with PBS (0.01 M, pH 7.4) at 2-8℃. Absorb the water with filter paper and weigh. Then add 9 times the volume of PBS according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, stored the serum at -80℃, which can be stored for a month. Meanwhile, determine the protein concentration of supernatant (A1034, A1035).

4. **Cell sample:**

   Collect the cells with cell scraper (Don't use trypsin or EDTA). Add PBS (0.01M, pH7.4) at a ratio of cell number (10^6): PBS (μL) =1: 300-500, then treat the sample with mechanical homogenate or sonication on ice. Centrifuge at 4ºC at 1500 g for 10 min and collect the supernatant for measurement. If not detected on the same day, stored the serum at-80℃, which can be stored for a month. Meanwhile, determine the protein concentration of supernatant (A1034, A1035).

5. **Saliva sample:**

   Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

6. **Urine samples:**

   Collect the fresh urine and centrifuge the sample at 10000 g for 10 min at 4℃. Take the supernatant and preserve it on ice for detection.

OPERATION STEPS

For serum (plasma) and other liquid samples

1. **Sample tube:** Add 1.0 mL of reagent 1 to 5 mL EP tube.
   Control tube: Add 1.0 mL of reagent 1 to 5 mL EP tube.
2. **Sample tube:** Add A* mL of sample to the tube. **Control tube:** Add nothing.
3. Add 2.0 mL of reagent 2 working solution and 0.5 mL of reagent 3 working solution to sample tube and control tube.
4. Mix fully and incubate the tubes at 37℃ for 30 min.
5. Add 0.1 mL of reagent 5 to sample tube and control tube.
6. **Sample tube:** Add nothing.
   Control tube: Add A* mL of sample to the tube.
7. Mix fully and stand for 10 min at room temperature. Set to zero with double-distilled water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz cuvette.

Note: It can be refer to the following operating table

	Sample tube	Control tube
Reagent 1 (mL)	1.0	1.0
Sample (mL)	A*	-
Reagent 2 working solution (mL)	2.0	2.0
Reagent 3 working solution (mL)	0.5	0.5
Mix fully and incubate the tubes at 37℃ for 30 min.		
Reagent 5 (mL)	0.1	0.1
Sample (mL)	-	A*
Mix fully and stand for 10 min at room temperature. Set to zero with double-distilled water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz cuvette.		

Note: For serum or plasma sample, it is recommended that A* is 0.1 mL.

For tissue and cells samples

1. **Sample tube:** Add 1.0 mL of reagent 1 to 5 mL EP tube. **Control tube:** Add 1.0 mL of reagent 1 to 5 mL EP tube.
2. **Sample tube:** Add A* mL of sample to the tube. **Control tube:** Add nothing.
3. Add 2.0 mL of reagent 2 working solution and 0.5 mL of reagent 3 working solution to sample tube and control tube.
4. Mix fully and incubate the tubes at 37℃ for 30 min.
5. Add 0.2 mL of reagent 5 to sample tube and control tube.
6. **Sample tube:** Add nothing **Control tube:** Add A* mL of sample to the tube.
7. Add 0.2 mL of reagent 6 to sample tube and control tube.
8. Mix fully and stand for 10 min at room temperature. Set to zero with double-distilled water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz cuvette.

Note: It can be refer to the following operating table.

	Sample tube	Control tube
Reagent 1 (mL)	1.0	1.0
Sample (mL)	A*	-
Reagent 2 working solution (mL)	2.0	2.0
Reagent 3 working solution (mL)	0.5	0.5
Mix fully and incubate the tubes at 37℃ for 30 min.		
Reagent 5 (mL)	0.2	0.2
Sample (mL)	-	A*
Reagent 6 (mL)	0.2	0.2
Mix fully and stand for 10 min at room temperature. Set to zero with double-distilled water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz cuvette.		

Note: It is recommended that A* is 0.1-0.2 mL.

Calculation of results

1. **Serum (plasma), whole blood and other liquid samples Definition:** At 37℃, the OD value of the reaction system was increased 0.01 by 1 mL of sample per minute is defined as a unit of total antioxidant capacity.

$$\text{T-AOC (U/mL)} = \frac{\Delta A}{0.01} \div 30^{*} \times \frac{V_1}{V_2} \times f$$

2. Tissue and cells sample:

Definition: At 37℃, the OD value of the reaction system was increased 0.01 by 1 mg of protein per minute is defined as a unit of total antioxidant capacity

$$\text{T-AOC (U/mgprot)} = \frac{\Delta A}{0.01} \div 30^{*} \times \frac{V_1}{V_2} \times f \div C_{pr}$$

Notes

ΔA1: OD_{sample}-$OD_{control}$

*: The reaction time, 30 min.

V_1: The total volume of reaction, mL.

V_2: The volume of sample added to the reaction, mL.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, mgprot/mL

TECHNICAL PARAMETER

1. The sensitivity of the kit is 0.62 U/mL.
2. The intra-assay CV is 2.7% and the inter-assay CV is 8.2%.
3. The recovery of the kit is 105%.
4. The detection range of the kit is 0.62-145.2 U/mL.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.