Total Antioxidant Capacity Assay Kit

Catalog No.: abx295022

Size: 96 tests (48 samples)

Detection Range: 0.62 U/ml - 190.43 U/ml

Sensitivity: 0.62 U/ml

Storage: Store all the kit components in the dark at 2-8°C.

Application: For detection and quantification of total antioxidant capacity (T-AOC) in serum, plasma, whole blood, tissue, cell lysates and cell culture supernatants.

Introduction

Total antioxidant capacity (TAC) determinations are simple, inexpensive, and able to evaluate the capacity of known and unknown antioxidants and their additive, synergistic and/or antagonistic actions, in chemical and biological systems. The TAC values for foods cannot be translated to the in vivo (human) antioxidant defenses, and furthermore, to health effects provided by that food. Alcoholism causes an impaired antioxidant capacity and a decreased secretion of amylase, which is ameliorated due to the alcohol withdrawal regimen. The strong correlation between blood and saliva with respect to the antioxidants suggests the potential future use of saliva as a laboratory tool in clinical medicine.

Abbexa's Total antioxidant capacity (TAC) Kit is a quick, convenient, and sensitive method for measuring and calculating total levels of antioxidant molecules and enzymes that reflects the total antioxidant capacity in the system. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm.

Kit components

1. 96-well Microplate
2. Buffer Solution: 12 ml
3. Chromogenic Reagent: 2 vials
4. Ferric Salt Stock Solution: 0.4 ml
5. Ferric Salt Diluent: 8 ml
6. Stop Solution: 2 × 1.25 ml
7. Clarificant: 2 × 1.25 ml
8. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (520 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Vials/tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Whole blood:** Collect fresh blood using heparin as the anticoagulant in a ratio 1:9. Keep on ice and assay immediately, or store at 2-8°C for 1-2 days.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 1500 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 1500 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.
- **Cell culture supernatant:** Collect cell culture supernatant into a centrifuge tube, then centrifuge at 3100 × g at 4°C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

Recommended dilution factor (for reference only):

Sample Type	Dilution Factor
Human Serum	1
Human Urine	1-2
10% Rat Liver Tissue Homogenate	1
HepG2 Cells	1
HepG2 Culture Supernatant	1
10% *Epipremnum aureum* Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Chromogenic Reagent working solution:** Dissolve a vial of Chromogenic Reagent with 20 ml double distilled water by incubating at 80-90°C in a water bath. Cool to room temperature before carrying out the assay.
- **Ferric Salt working solution:** Dilute Ferric Salt Stock Solution with Ferric Salt Diluent at a ratio of 1:19. Prepare immediately before carrying out the assay.
- **Clarificant working solution:** Incubate the Clarificant at 37°C before use. Prepare immediately before carrying out the assay.

B. Assay Procedure

1. Serum and Plasma

1.1. Set the Sample and Control tubes for each sample.
1.2. Add 100 µl of Buffer Solution to each Sample and Control tube.
1.3. Add 10 µl of sample to the Sample tubes.
1.4. Add 200 µl of Chromogenic Reagent working solution and 50 µl of Ferric Salt working solution to each Sample and Control tube.
1.5. For each tube, mix fully and incubate at 37°C in water bath for 30 minutes.
1.6. Add 10 µl of Stop Solution to each Sample and Control tube.
1.7. Add 10 µl of sample to the Control tubes.
1.8. For each tube, mix fully and allow to stand at room temperature for 10 minutes.
1.9. Set the Sample and Control wells on the well-plate, add 300 µl of reaction solution to each well.
1.10. Measure the OD values at 520 nm with a microplate reader.

2. Tissue and Cells Samples

2.1. Set the Sample and Control tubes for each sample.
2.2. Add 100 µl of Buffer Solution to each Sample and Control tube.
2.3. Add 10 µl of sample to the Sample tubes.
2.4. Add 200 µl of Chromogenic Reagent working solution and 50 µl of Ferric Salt working solution to each Sample and Control tube.
2.5. For each tube, mix fully and incubate at 37°C in water bath for 30 minutes.
2.6. Add 20 µl of Stop Solution to each Sample and Control tube.
2.7. Add 10 µl of sample to the Control tubes.
2.8. Add 20 µl of Clarificant working solution to each Sample and Control tube.
2.9. For each tube, mix fully and allow to stand at room temperature for 10 minutes.
2.10. Set the Sample and Control wells on the well-plate, add 300 µl of reaction solution to each well.
2.11. Measure the OD values at 520 nm with a microplate reader.

3. Whole Blood Samples

3.1 Set the Sample and Control tubes for each sample.
3.2 Add 100 µl of Buffer Solution to each Sample and Control tube.
3.3 Add 10 µl of sample to the Sample tubes.
3.4 Add 200 µl of Chromogenic Reagent working solution and 50 µl of Ferric Salt working solution to each Sample and Control tube.
3.5 For each tube, mix fully and incubate at 37°C in water bath for 30 minutes.
3.6 Add 20 µl of Stop Solution to each Sample and Control tube.
3.7 Add 10 µl of sample to the Control tubes.
3.8 For each tube, mix fully and allow to stand at room temperature for 10 minutes.
3.9 Set the Sample and Control wells on the well-plate, add 300 µl of reaction solution to each well.
3.10 Measure the OD values at 520 nm with a microplate reader.

C. Calculation of Results

1. Serum, plasma, whole blood samples:

One unit of total antioxidant capacity is defined as the quantity of total antioxidant system increased by 1 ml of sample in 1 minute at 37°C that is equivalent to the 0.01 increase of total antioxidant concentration.

$$\mathrm{T-AOC\ (U/ml)} = \frac{\Delta A_{520}}{0.01 \times T} \times \frac{V_1}{V_2} \times f$$

2. Tissues and cell samples:

One unit of total antioxidant capacity is defined as the quantity of total antioxidant system increased by 1 mg of protein in 1 minute at 37°C that is equivalent to the 0.01 increase of total antioxidant concentration.

$$\mathrm{T-AOC\ (U/mg\ protein)} = \frac{\Delta A_{520}}{0.01 \times T} \times \frac{V_1}{V_2} \times \frac{f}{C_P}$$

where:

ΔA_{520}	OD value of the sample ($OD_{Sample} - OD_{Control}$)
f	dilution factor of the sample before carrying out the assay
V_1	total volume of reaction
V_2	volume of sample added to the reaction
T	reaction time (30 minutes)
C_P	concentration of protein in sample (mg protein/ml)