Cat. No. 299

Fe-TAC®

Total Antioxidant Capacity (TAC) High Sensitivity Colorimetric Assay 250 assays

Quick User Guide

1. Storage conditions before opening: refrigerated at 4°C for half a year.
2. Absorption spectrometer detection wavelength (nm): 595 nm.
3. Standard or sample volume: 100 μL.
4. The volume ratio of working solution：reaction solution (agent A), iron (III) chloride solution (agent B) and Assay buffer solution (agent C) = 1:1:8.
5. Reaction solution addition amount: 100 μL.
6. Reaction temperature and time: 15 minutes at room temperature.
7. Working range：0~200 μM.

Introduction

Total Antioxidant Capacity (TAC) can represent the antioxidant capacity in the body. Antioxidants play an important role in preventing the formation and scavenging of free radicals and other potentially toxic oxidizing substances. Antioxidants can be divided into three categories: enzyme systems (GSH reductase, catalase, peroxidase, etc.), small molecules (ascorbic acid, uric acid, vitamin E, etc.) and proteins (albumin, transferrin, etc.). The antioxidant capacity of different antioxidants varies. The detection of TAC in the sample can reflect the antioxidant status of the sample in the physiological environment more than the measurement of a certain antioxidant alone. Studies have shown that TAC is significantly lower in patients with cancer, diabetes and myocardial infarction than normal. They Prove that oxidative stress is an important factor of disease. The measurement results of this set can directly reflect the influence of reactive oxygen species (ROS) in the sample, including the state of resistance to oxidative damage and oxidative-related diseases.

After removing the interference of GSH and protein signals, this set can measure the antioxidant power of sample by reducing Fe^{3+} to Fe^{2+}. It will produce a strong absorption spectrum change near the 595 nm spectrum which can be quantitatively detected after Fe^{2+} chelates with the color-forming agent. By comparing the OD_{595} of the sample with the ascorbic acid standard, the TAC value in the sample can be expressed by ascorbic acid equivalents. This set can use a 96-well plate with a microplate reader or use a 0.5 mL cuvette with the recommended spectrometer for measurement. Please refer to the operation steps for the operation method.

Kit Components and Reagent Preparation

1. Reaction solution (agent A): 3 mL brown glass bottle*1, used directly.
2. Iron(III) chloride solution (agent B): 3 mL translucent plastic tube*1, used directly.
3. 5X Assay buffer (agent C): 25 mL white plastic bottle*1, diluted to 1X with ddH_2O before use.
4. Ascorbic acid standard: 200 mg powder in brown pointed bottom plastic bottle*1, add 2 mL ddH_2O into 35 mg ascorbic acid powder to get 10X ascorbic acid standard solution (100 mM). The standards can be used immediately or divided into portions after reconstitution. Ascorbic acid solution should be stored at -20°C in 100 μL.

Package and Shipping information

Composition	Shipping Information	Store information before opening the package
1. Agent A: reaction solution	Refrigerate at 4 °C.	Refrigerate at 4 °C. Under proper storage conditions, it can be stored for half a year.
2. Agent B: Iron (III) chloride solution		
3. Agent C:5X Assay buffer		
4. Ascorbic acid standard		

Materials Not Supplied

1. 96-well microtiter plates
2. 10 kDa MWCO centrifugal filter (for high protein content samples)
3. Distillation-Distillation H_2O (ddH_2O)
4. Micropipettes
5. Conical tubes, microcentrifuge tubes, and bottles for sample and buffer preparation
6. Centrifuge and/or microfuge
7. Sonicator or tissue homogenizer
8. Microplate reader
9. Phosphate buffered saline (PBS)

Preparation of Samples

All samples should be analyzed immediately or stored for up to 2 months at -80°C after sampling. The test reading should be within the linear confidence interval of the standard. The high concentration of the sample may cause the interference by other components in the sample. Recommend to dilute with PBS as needed before testing. Always run a standard curve with samples.

Body fluids: Centrifuge tissue fluid at 12,000 rpm for 20 minutes at 4°C. After extracting the supernatant, samples should be tested immediately or frozen at -80°C.

Blood: Blood samples were collected with heparin tube* as anticoagulant. Centrifuge at 3,000 rpm for 10-15 minutes at 4°C. Carefully aspirate the upper yellow plasma supernatant. Samples should be tested immediately or frozen at -80˚C.

Note: Hemolysis should be avoided. Limited use of heparin as an anticoagulant, EDTA will interfere with the color reaction.

Cell Lysates: Lyse 1-2 x 10^6 cells/mL by sonication or multiple freeze-thaw cycles in 4 volumes of cold 1X Assay Buffer. Centrifuge at 12,000 rpm for 15 minutes at 4ºC and remove insoluble cell material. A high concentration of protein may interfere with the assay. Filter the sample with a 10kDa MWCO centrifugal filter before assaying (to remove protein interference). Samples should be tested immediately or frozen at -80˚C.

Tissue Lysates: 10 mg of tissue was homogenized/sonicated with 1~2 mL of 1X Assay Buffer (five-fold dilution of agent C) at 4°C. The supernatant was collected after centrifugation of the homogenate at 12,000 rpm for 15 min at 4°C. High concentrations of protein may interfere with the assay. 10 kDa protein centrifuge filter tubes can be used to exclude protein interference. Test the samples immediately or store it at -80°C.

Food Extracts: Wash uncooked food (e.g. fruits or vegetables) in water and homogenize 5g (fresh wet weight) in 100 mL distilled water for 30 seconds. Centrifuge the homogenate at 12,000 rpm for 15 min at 4°C and collect the supernatant. After extracting the supernatant, samples should be tested immediately or frozen at -80°C.

Assay Protocol and Preparation of Standard Curve

1. Thaw 10X ascorbic acid standard (100 mM) and dilute 10-fold with 1X Assay buffer. Serial dilutions of ascorbic acid standards (10 mM) were made to configure a standard curve. The configuration can refer to the following table:

Standard Tubes	Concentration (μM)	Source (μL)	1X Assay Buffer (μL)	Total volume (μL)
A	200	40 from ascorbic acid standard (10mM)	1960	2000
B	100	750 from A tube	750	1500
C	50	750 from B tube	750	1500
D	25	750 from C tube	750	1500
E	20	100 from A tube	900	1000
F	10	100 from B tube	900	1000
G	2	100 from E tube	900	1000
H	1	100 from F tube	900	1000
I	0 (Blank)	--	1000	1000

2. Prepare the working solution: Mix the reaction solution(agent A), the iron (III) chloride solution (agent B) and 1X Assay buffer solution (diluted agent C) in a volume ratio of **1：1：8**.
   (For example: 100 mL of working solution = 10 mL of agent A + 10 mL of agent B + 80 mL 1X Assay buffer solution).
3. Add 100 μL of gradient concentration standard solution or sample to 96-well plate.
4. Add 100 μL of the working solution and incubate the plate for 15 minutes at room temperature. Keep out of light during the reaction.
5. Read absorbance on a microplate reader using 595 nm as the dominant wavelength. Make sure the instrument reading is within the linear range of the assay (0-2.5 OD), and dilute the sample if necessary.

Calculation of Results

1. A standard curve was drawn by subtracting the background value (Blank) from the mean absorbance value of the ascorbic acid standard (Figure 1).
2. Calculate the ascorbic acid equivalents of the TAC of the sample by subtracting the background value from the average absorbance value and comparing it with the standard curve (Figure 2).

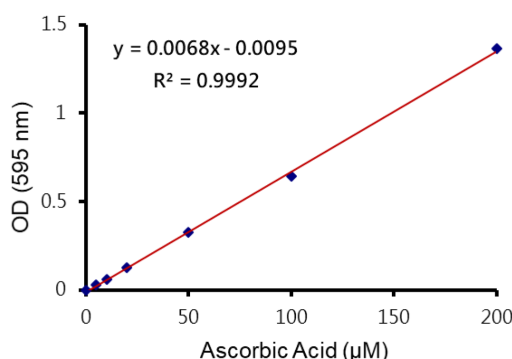
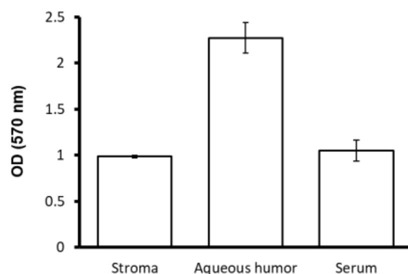


Figure 1. Ascorbic Acid Standard Curve

(A).



(B).

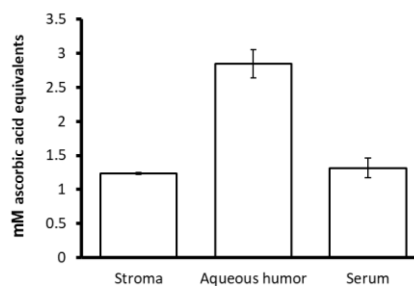


Figure 2. Example of TAC quantization scaling. (A). Results from the TAC analysis can be presented as OD_{570} readings. (B). The TAC of the samples is presented in ascorbic acid equivalent values to allow comparisons between different experiments.

References

1. Tsao, Yu-Ting, et al., *Bioeng Transl Med.* **2020**,6(2), e10199.

2. Tsao, Yu-Ting, et al., *Antioxidants* **2022**, *11*, 397.

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