

**Total Antioxidant Capacity (TAC) Broad Range 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): 570 nm.
3. Standard or sample volume: 10 µL.
4. The volume ratio of reaction solution colorant (agent A) and copper sulfate solution (agent B): 25:2.
5. Reaction solution addition amount: 200 µL.
6. Reaction temperature and time: 15 minutes at room temperature.
7. Working range : 50~2000 µg/mL °

**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  $\text{Cu}^{2+}$  to  $\text{Cu}^+$ . It will produce a strong absorption spectrum change near the 570 nm spectrum which can be quantitatively detected after  $\text{Cu}^+$  chelates with the color-forming agent. By comparing the  $\text{OD}_{570}$  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. Color former (agent A): 50 mL brown plastic bottle\*1, used directly.
2. Copper sulfate solution (agent B): 4 mL translucent plastic tube\*1, used directly.
3. 5X Assay buffer (agent C): 50 mL white plastic bottle\*1, diluted to 1X with ddH<sub>2</sub>O before use.
4. Ascorbic acid standard: 1 mL brown pointed bottom plastic bottle\*1, add 1 mL

ddH<sub>2</sub>O 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.

5. Protein blocking agent (agent D): 4 mL translucent plastic tube\*1.

### **Package and Shipping information**

<b>Composition</b>	<b>Shipping Information</b>	<b>Store information before opening the package</b>
1. Agent A: color former	Refrigerate at 4 °C.	Refrigerate at 4 °C. Under proper storage conditions, it can be stored for half a year.
2. B agent: copper sulfate solution		
3. Agent C: 5X buffer solution		
4. Ascorbic Acid Standard		
5. Agent D: protein blocking agent		

### **Materials Not Supplied**

1. 96-well microtiter plates
2. 10 kDa MWCO centrifugal filter (for high protein content samples)
3. Distillation-Distillation H<sub>2</sub>O (ddH<sub>2</sub>O)
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<sup>6</sup> 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 4°C 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. After thawing the sample and adding half the volume of the protein blocker solution to the sample, incubate the plate at room temperature for 15 minutes. Centrifuge at 12,000 rpm for 15 minutes before collect the supernatant. (e.g. 10 µL sample with 5 µL blocking reagent)
2. 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 (mM)	Source (µL)	1X Assay Buffer (µL)	Total volume (µL)	final volume (µL)
A	2.5	<sup>125</sup> 10 mM ascorbic acid standard	375	500	500
B	2	<sup>200</sup> 10 mM ascorbic acid standard	800	1000	1000
C	1.5	<sup>150</sup> 10 mM ascorbic acid standard	850	1000	800
D	1	<sup>90</sup> 10 mM ascorbic acid standard	810	900	400
E	0.75	200 from C tube	200	400	400
F	0.5	500 from D tube	500	1000	500
G	0.25	400 from F tube	400	800	800
H	0.05	100 from F tube	900	1000	1000
I	0 (Blank)	--	500	500	500

3. Prepare the reaction solution: Mix the color former (agent A) and the copper sulfate solution (agent B) in a volume ratio of 25:2.  
(For example: 27 mL of reaction solution = 2 mL of copper sulfate solution + 25 mL of agent A).
4. Add 10 µL of gradient concentration standard solution or sample to 96-well plate.
5. Add 200 µL of the reaction reagent and incubate the plate for 15 minutes at room temperature. Keep out of light during the reaction.
6. Read absorbance on a microplate reader using 570 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).

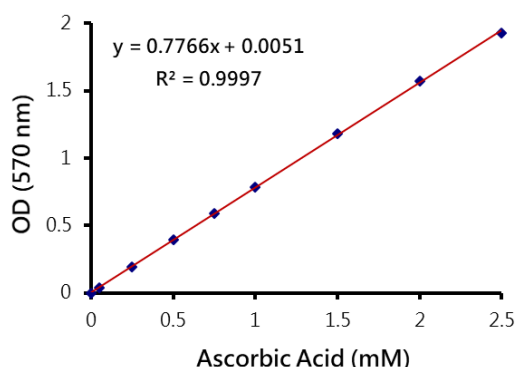


Figure 1. Ascorbic Acid Standard Curve

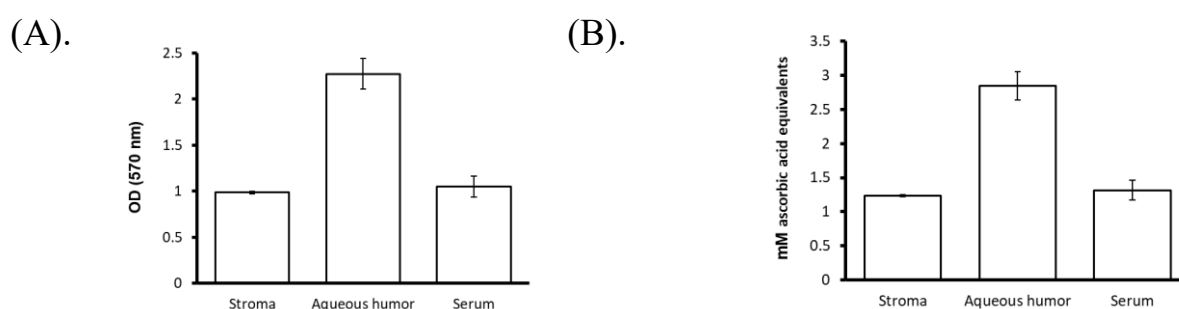


Figure 2. Example of TAC quantization scaling.

(A). Results from the TAC analysis can be presented as OD<sub>570</sub> 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.

---

聚創新材料股份有限公司

IMT FORMOSA New Materials Co., Ltd.

Phone: 0926-159317

Email: [polycreatives@outlook.com](mailto:polycreatives@outlook.com)

Contact: Peter Hsu

