



Total Antioxidant Capacity Microplate Assay Kit User Manual

Catalog # CAK1111

(Version 1.3D)

Detection and Quantification of Total Antioxidant Capacity (TAC)
Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell
culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants protect the cells from damages by reactive oxygen species which are produced in oxidation reactions in the cell. Antioxidants can be small molecules such as glutathione, vitamins, or macromolecules such as catalase, glutathione peroxidase. As oxidative stress contributes to the development of many diseases including Alzheimer's disease, Parkinson's disease, diabetes, rheumatoid arthritis and neurodegeneration, the use of antioxidants in pharmacology is intensively studied. Antioxidants are also widely used as dietary supplements and in industry as preservatives in food, cosmetics, rubber and gasoline.

The assay measures total antioxidant capacity in which Fe^{3+} -TPTZ is reduced by antioxidant to Fe^{2+} -TPTZ. The enzyme catalysed reaction products Fe^{2+} -TPTZ can be measured at a colorimetric readout at 593 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	16 ml x 1	4 °C
Substrate	Powder x 1	4 °C, keep in dark
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	2 ml x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Standard: add 1 ml distilled water to dissolve, mix. Then add 0.1 ml into 0.9 ml distilled water, mix. The concentration is 5 mmol/L, store at 4 °C.

Substrate: add 1.5 ml distilled water to dissolve before use.

Dye Reagent: add 2 ml Dye Reagent Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 593 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 12,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum, plasma or urine samples

Detect directly.

Note: Serum, plasma can not use EDTA as the anticoagulant. The sample also can not contain DTT, Mercaptoethanol, Tween, Triton, NP-40.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	160 μ l	160 μ l	160 μ l
Sample	5 μ l	--	--
Standard	--	5 μ l	--
Distilled water	--	--	5 μ l
Dye Reagent	20 μ l	20 μ l	20 μ l
Mix.			
Substrate	15 μ l	15 μ l	15 μ l
Wait for 5 minutes, measured at 593 nm and record the absorbance.			

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Total Antioxidant Capacity is defined as the sample generates 1 μmol of Fe^{2+} per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{TAC (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad C_{\text{Protein}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{TAC (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{TAC (U/10}^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of serum, plasma

$$\begin{aligned} \text{TAC (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

C_{Protein} : the protein concentration, mg/ml;

C_{Standard} : the standard concentration, 5 mmol/L = 5 $\mu\text{mol/ml}$;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Standard} : the volume of standard, 0.005 ml;

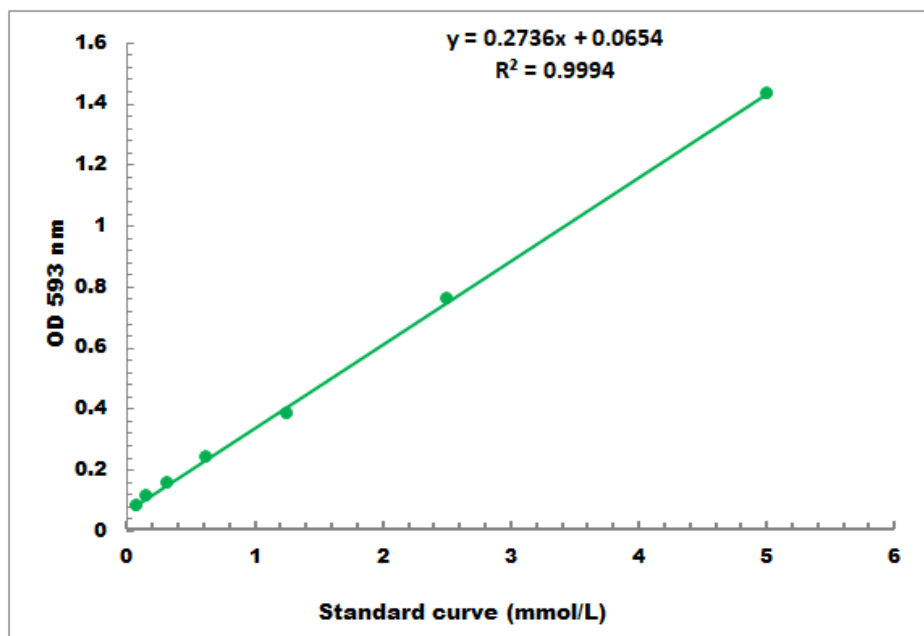
V_{Sample} : the volume of sample, 0.005 ml;

V_{Assay} : the volume of Assay buffer, 1 ml.

T: the reaction time, 5 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES