



Total Antioxidant Capacity Microplate Assay Kit User Manual

Catalog # ASK1111

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 |
| Technical Manual | 1 Manual | |

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
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%, sonication 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. | | | |

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

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

2. According to the weight of sample

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

3. According to the quantity of cells or bacteria

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

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 total volume of the reaction, 0.005 ml;

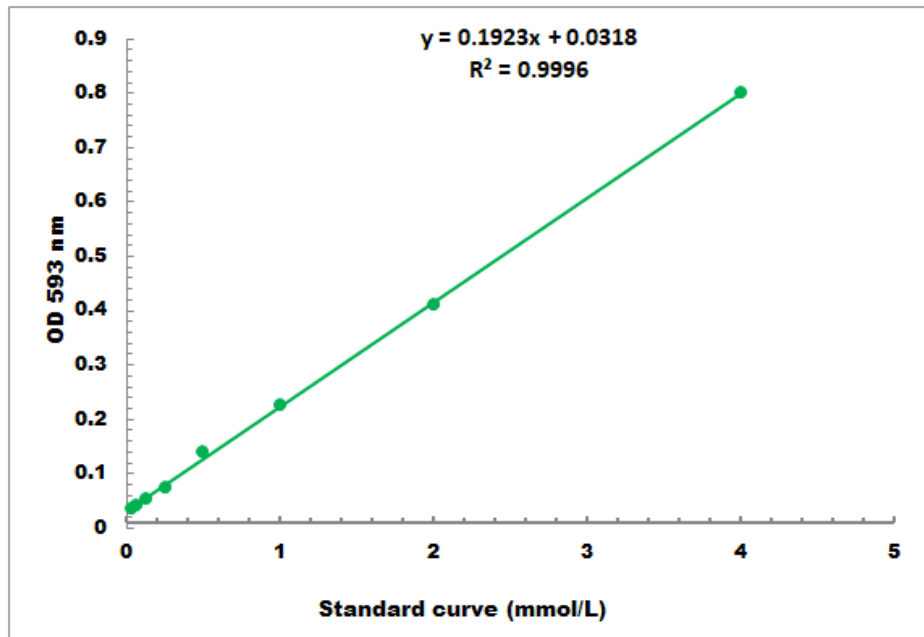
V_{Sample} : the volume of sample, 0.005 ml;

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