Total Antioxidant Capacity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1111

(Version 1.5D)

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.

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.

Total Antioxidant Capacity Colorimetric Microplate Assay Kitprovides a convenient tool to measure total antioxidant capacity in which Fe^{3+}-TPTZ is reduced by antioxidant toFe^{2+}-TPTZ. The enzyme catalysed reaction products Fe^{2+}-TPTZcan be measured at a colorimetric readout at 593 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	16 mlx 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 mlx 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

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

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 50 mmol/L of standardstock solution.Store at 4°C for 1 day or -20 °C for 1 week after reconstitution.Then dilute to5 mmol/Lstandard top solutionby adding 0.1 mlstock solution into 0.9 ml distilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be5/2.5/1.25/0.625/0.312/0.156/0.078mmol/L.Prepare working solutionfresh for immediate use.

Substrate: Briefly centrifuge prior to opening. Add 1.5 mldistilled water to dissolve before use. Keep in dark and store at 4°C for 3-4 days or-20 °C for 1-2 weeks.

Dye Reagent: Briefly centrifuge prior to opening. Add 2 mlDye Reagent Diluent to dissolve before use.Keep in dark and store at 4°C for 1 month or -20 °C for 3 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

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

3.For serum,plasmaor urinesamples

Detect directly.

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

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	160 µl	160 µl	160 µl
Sample	5 µl	--	--
Standard	--	5 µl	--
Distilled water	--	--	5 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix.			
Substrate	15 µl	15 µl	15 µl
Wait for 5 minutes, measured at 593 nm and recordthe absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VI. CALCULATION

Unit Definition: One unit of Total Antioxidant Capacityis defined asthe sample generates 1μmol of Fe^{2+}per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.2According to the quantity of cells or bacteria

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times N\times(V_{Sample}/ V_{Assay})\times T}(U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times W \times(V_{Sample}/ V_{Assay})\times T}(U/g)$$

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

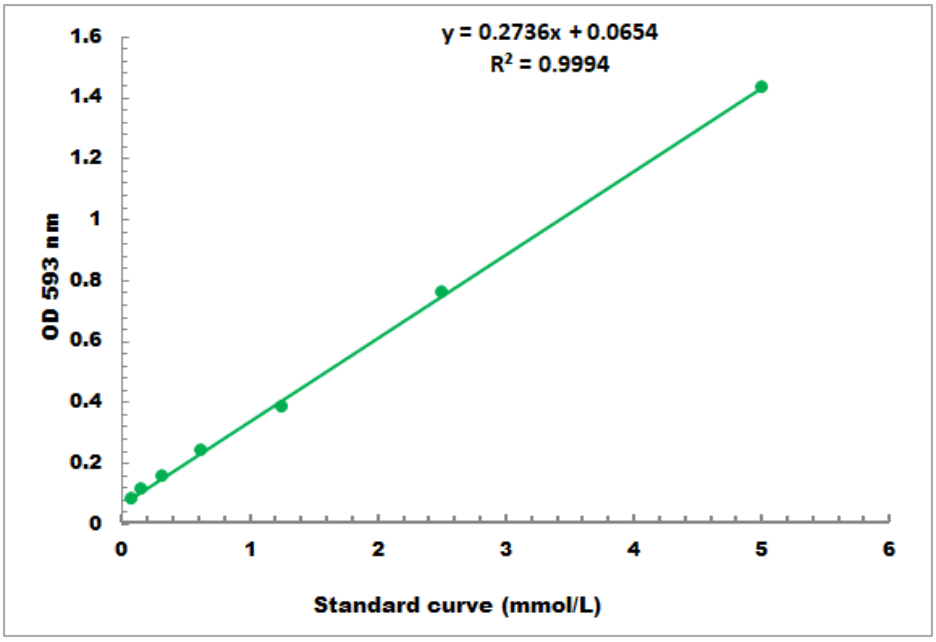
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. 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