CheKine™ Total Antioxidant Capacity (TAC) Assay Kit

Item NO.	**Product Name**
KTB1500	CheKine™ Total Antioxidant Capacity (TAC) Assay Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201904

Abbkine, Inc • China • ***www.abbkine.com***

Not for further distribution without written consent. Copyright © Abbkine, Inc.

TABLE OF CONTENTS

INTRODUCTION

Background..1
Assay principles..1
Storage/Stability..1
Assay restrictions...1

PRODUCT INFORMATION

Materials supplied and Storage conditions...2
Other supplies required, Not Supplied...2
Technical hints...2

ASSAY PROTOCOL

Reagent preparation...3
Standard preparation..3
Sample preparation...4
Assay procedure..4

DATA ANALYSIS

Calculation of results..5
Typical data...6

Not for further distribution without written consent. Copyright © Abbkine, Inc.

INTRODUCTION

Background

Antioxidants play an important role in preventing the formation of and scavenging of free radicals and other potentially toxic oxidizing species. There are three categories of antioxidant species: enzyme systems (GSH reductase, catalase, peroxidase, etc.), small molecules (ascorbate, uric acid, GSH, vitamin E, etc.) and proteins (albumin, transferrin, etc.). 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.

Assay principle

Abbkine Total Antioxidant Capacity Assay Kit provides a simple and easy colorimetric assay for measuring Total Antioxidant Capacity in serum, plasma, cell culture supernatants, urine, tissue/ cell lysates and other biological fluids. In this assay, Fe^{3+}-TPTZ is reduced by antioxidant to Fe^{2+}-TPTZ. The enzyme catalyzed reaction products Fe^{2+}-TPTZ can be measured at a colorimetric readout at 593 nm.

Storage/Stability

Storage at -20°C and Keep from light. Stable for at least 12 months at recommended temperature from date of shipment.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Not for further distribution without written consent. Copyright © Abbkine, Inc.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity			Storage conditions
	48T	96T	480T	
Assay Buffer (10X)	6 mL	12 mL	60 mL	4ºC
Substrate Diluent	10 mL	20 mL	100 mL	4ºC
Substrate	1 mL	2 mL	10 mL	-20ºC, protect from light
Reaction Buffer	1 mL	2 mL	10 mL	-20ºC, protect from light
$FeSO_4$• $7H_2O$ Standard	100 mg	200 mg	1g	4ºC, protect from light
ascorbic acid (positive control)	1 mg	1 mg	1 mg	4ºC

Other supplies required, Not Supplied

- Standard microplate reader capable of measuring absorbance at 593 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (for tissue samples)

Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

Not for further distribution without written consent. Copyright © Abbkine, Inc.

ASSAY PROTOCOL

Reagent preparation

Assay Buffer (10X): Equilibrate to room temperature before use. Make a 1:10 dilution of the concentrated Assay Buffer with distilled or deionized water. When stored at 4 °C, this diluted Assay Buffer is stable for at least two months.
Substrate Diluent, Substrate, Reaction Buffer: Ready to use as supplied. Store at -20°C and protect from light.
ascorbic acid (positive control): add 1 mL diluted Assay Buffer to dissolve, mix. Then add 0.1 mL into 0.9 mL diluted Assay Buffer, mix. The concentration is 0.1 mg/mL, and store at 4 °C.

Standard preparation

- Always prepare a fresh set of standards per use.
- Diluted standard solution is unstable and must be used within 4 hours

Dissolve 27.8 mg $FeSO_4 \cdot 7H_2O$ with 1mL Assay Buffer to prepare 100 mM standard. Prepare 3 mM of Standard by diluting 18 µL 100mM Standard into 582 µL Assay Buffer. Using 3 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

	Volume of 20 µM Standard	Assay Buffer（1X)	Concentration
Std.1	200 µL	0 µL	3 mM
Std.2	160 µL	40 µL	2.4 mM
Std.3	120 µL	80 µL	1.8 mM
Std.4	80 µL	120 µL	1.2 mM
Std.5	40 µL	160 µL	0.6 mM
Std.6	20 µL	180 µL	0.3 mM
Std.7	10 µL	190 µL	0.15 mM
Blank	0	200 µL	0

Note: If sample is cell culture supernatant, please prepare Standards with culture medium.

Not for further distribution without written consent. Copyright © Abbkine, Inc.

Sample preparation

Note: We recommend performing several dilutions of your sample to ensure the readings are within the standard value range. We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C for one month. Can not use EDTA as the anticoagulant for plasma samples. The samples also can not contain DTT, Mercaptoethanol, Tween, Triton, NP-40.

Cell and bacteria samples: Collect cell or bacteria into centrifuge tube, wash cell or bacteria with cold PBS. Add 1mL Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.
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.
Serum, plasma or urine samples: Detect directly.

Assay procedure

Add following reagents into the microplate:

Reagent	Sample	Standard	positive control
Substrate Diluent	150 μL	150 μL	150 μL
Substrate	15 μL	15 μL	15 μL
Mix.			
Reaction Buffer	15 μL	15 μL	15 μL
Sample	10 μL	--	--
Standard	--	10 μL	--
positive control	--	--	10 μL
Incubate 5 minutes at room temperature. Measure the absorbance at 593 nm.			

Not for further distribution without written consent. Copyright © Abbkine, Inc.

DATA ANALYSIS

Calculation of results

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

1. According to the protein concentration of sample

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

$= 0.6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$

2. According to the weight of sample

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

$= 0.6 \times (ODSample - ODBlank) / (ODStandard - ODBlank) / W$

3. According to the quantity of cells or bacteria

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

$= 0.6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

4. According to the volume of serum, plasma

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

$= 0.6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

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

$C_{Standard}$: the standard1 concentration, 3 mmol/L = 3 μmol/mL;

W: the weight of sample, g;

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

$V_{Standard}$: the volume of the standard, 0.01 mL;

V_{Sample}: the volume of sample, 0.01 mL;

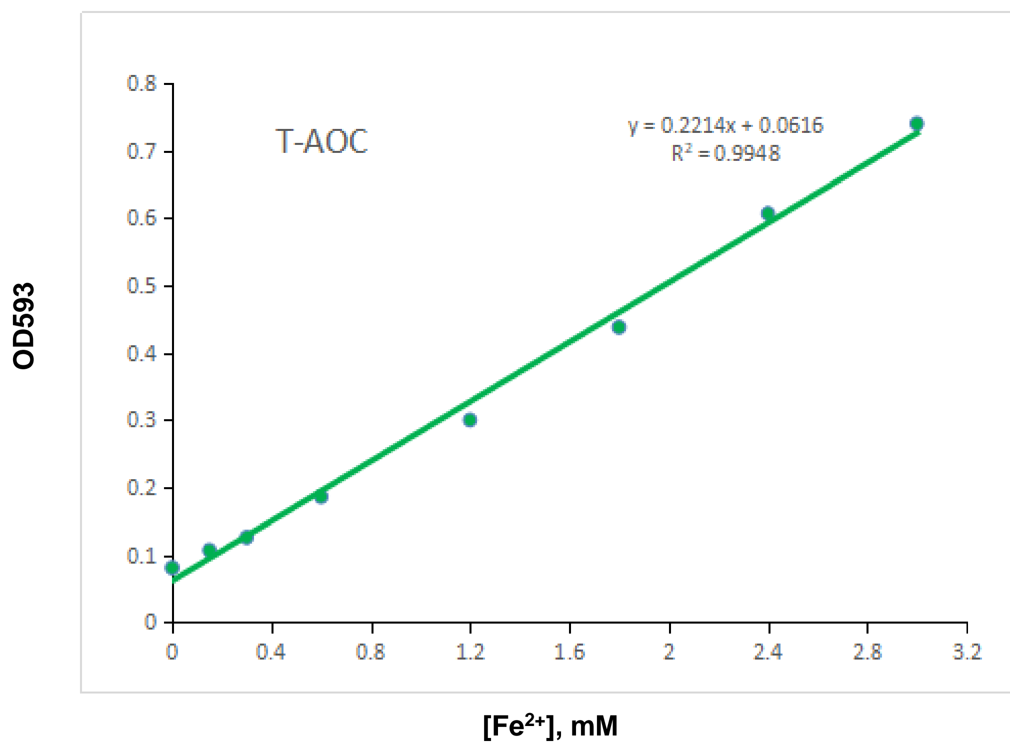
V_{Assay}: the volume of Assay buffer, 1 mL.

T: the reaction time, 5 minutes.

Not for further distribution without written consent. Copyright © Abbkine, Inc.

Typical data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Abbkine, Inc • China • ***www.abbkine.com***
Not for further distribution without written consent. Copyright © Abbkine, Inc.