CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit

Cat #: KTB1500 Size: 96 T/480 T

	Micro Total Antioxidant Capacity (TAC) Assay Kit		
REF	**Cat #:** KTB1500	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria, Urine		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Antioxidants play an important role in preventing the formation 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. CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit provides a convenient tool for detection of Total Antioxidant Capacity (TAC) in serum, plasma, animal and plant tissues, cells, bacteria, urine and other biological fluids. The principle is that in an acidic environment, antioxidants can reduce Fe^{3+}-Tripyridine triazine (Fe^{3+}-TPTZ) to produce blue Fe^{2+}-TPTZ, and the total antioxidant capacity of samples was obtained by determining the content of Fe^{2+}-TPTZ at 593 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Assay Buffer (10×)	12 mL	60 mL	4℃
Substrate Diluent	20 mL	100 mL	4℃
Substrate	2 mL	10 mL	-20℃, protected from light
Reaction Buffer	2 mL	10 mL	-20℃, protected from light
Ascorbic Acid (Positive Control)	1 mg	1 mg	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 593 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

1×Assay Buffer: Dilute Assay Buffer (10×) with deionized water to 1×Assay Buffer before use. 1×Assay Buffer can be stored at 4℃ at least 2 months.

Substrate Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Substrate: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from ligh after aliquoting to avoid repeated freezing and thawing.

Reaction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from ligh after aliquoting to avoid repeated freezing and thawing.

Working Ascorbic Acid (Positive Control): Add 1 mL 1×Assay Buffer to dissolve before use. Then add 0.1 mL this solution to 0.9 mL 1×Assay Buffer and mix well. The concentration is 0.1 mg/mL, stored at 4℃, protected from light.

Working Reagent: Prepare according to the ratio as Substrate Diluent: Substrate: Reaction Buffer =10:1:1 before use. Working Reagent is freshly prepared.

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for one month. EDTA cannot be used as an anticoagulant for plasma samples. The sample should not contain detergents such as DTT, mercaptoethanol, Tween, Triton, and NP-40, and reducing agents such as DTT and mercaptoethanol that affect the redox reaction.

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL 1×Assay Buffer and homogenize on ice. Transfer to 1.5 mL EP tubes, centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissues: Weigh 0.1 g tissue, add 1 mL 1×Assay Buffer and mash. Ultrasonic break on ice 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Transfer to 1.5 mL EP tubes, centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL 1×Assay Buffer to ultrasonically disrupt the cells or bacteria on ice 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Transfer to 1.5 mL EP tubes, centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Serum, Plasma or Urine: Tested directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 593 nm, Visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each well in 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Test Well (μL)	Positive Control Well (μL)
Deionized Water	10	0	0
Sample	0	10	0
Working Ascorbic Acid (Positive Control)	0	0	10
Working Reagent	180	180	180

3. Mix well, and place in room temperature for 5 min, then reading the values at 593 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$. (Only one blank well needs to be detected)

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A_{Test} is greater than 1.5, the sample can be appropriately diluted with 1×Assay Buffer, the calculated result multiplied by the dilution factor.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Standard curve Calculation formula of TAC: y=4.4664x+0.0685, R^2=0.9986, y is Fe^{2+} concentration (μmol/mL), x is the absorbance change value (ΔA), Substitute the ΔA_{Test} into the equation to obtain the y value (μmoL/mL).

2. Calculation TAC of samples

Unit Definition: The TAC of the sample is defined as the standard solution Fe^{2+} concentration (μmol/mL) required to achieve the same absorbance change value (ΔA).

(1) Calculated by sample fresh weight

TAC (μmol/g fresh weight)=$y\times V_{Reaction\ Total}\div(V_{sample}\div V_{sample\ Total}\times W)\times n$**=19×y÷W×n**

(2) Calculated by protein concentration

TAC (μmol/mg prot)=$y\times V_{Reaction\ Total}\div(V_{sample}\times Cpr)\times n$**=19×y÷Cpr×n**

(3) Calculated by cells or bacteria number

TAC (μmol/10^4)=$y\times V_{Reaction\ Total}\div V_{sample}\times V_{sample\ Total}\div$cells or bacteria number×n**=19×y÷cells or bacteria number×n**

(4) Calculated by liquid volume

TAC (μmol/mL)=$y\times V_{Reaction\ Total}\div V_{sample}\times n$**=19×y×n**

Where: $V_{Reaction\ Total}$: total reaction volume,0.19 mL; V_{sample}: sample volume added, 0.01 mL; $V_{sample\ Total}$: Extraction Buffer volume added, 1 mL; W: sample weight, g; n: dilution multiple of sample further dilution; Cpr: sample protein concentration, mg/mL; Cells or bacteria number: 10^4 as the unit.

Note: it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Typical Data

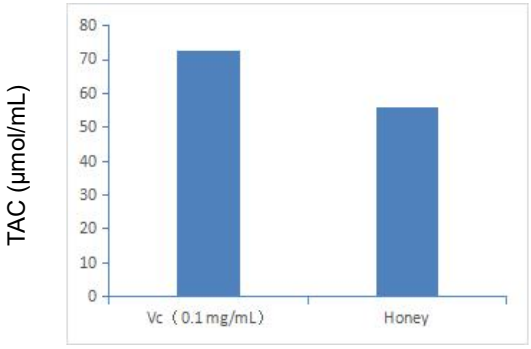

Figure 1. TAC in Vc (0.1 mg/mL) and Honey respectively.

Recommended Products

Catalog No.	Product Name
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit
KTB1091	CheKine™ Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit
KTB1550	CheKine™ Micro Ceruloplasmin Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.