

GENCHEM® Total Antioxidant Capacity (T-AOC) (Colorimetric) Assay Kit

REF : KBCA2221

Ver 1.1

RUO

Biochemical Assay for the Quantitative Determination of Total Antioxidant Capacity (T-AOC) in Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria, Urine

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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 96 tests

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Introduction:

The GENCHEM® Assay kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma, tissue cells and cell culture supernatant as validated with the kit. The kit employs in vitro quantitative determination of Lactate Dehydrogenase in the sample.

Intended Use:

The GENCHEM® Total Antioxidant Capacity (T-AOC) (Colorimetric) Assay kit is used as an analytical tool for quantitative determination of Total Antioxidant Capacity (T-AOC) in Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria, Urine.

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. 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 Provided:

1. Extraction Buffer – 120 ml
2. Standard (powder) – 2 vials
3. Reagent I – 40 ml
4. Reagent II – 2 ml
5. Reagent III – 2 ml
6. Standard Diluent – 10 ml
7. Positive Control (powder) – 1 mg

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Clean tubes and Eppendorf tubes.
6. Precision single and multi-channel pipette and disposable tips.

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. Storage condition for each component has been mentioned on the label.
3. All the reagents should be used within 12 months from manufacturing date.
4. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.



Sample preparation:

1. Animal Tissues: Weigh 0.1 g tissue, add 1ml Assay Buffer and homogenize on ice. Transfer to 1.5ml EP tubes, centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. Plant Tissues: Weigh 0.1 g tissue, add 1ml 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 1.5ml EP tubes, centrifuge at 10,000 g for 10 min at 4°C. 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 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°C. Use supernatant for assay, and place it on ice to be tested.
4. Liquid samples: Test directly. If turbid, centrifuge and collect the supernatant. Determine the dilution factor based on preliminary experiments.
5. Drugs: May be prepared at a certain concentration use the extraction buffer, 0.5 mg/ml.

Reagent preparation:

Positive Control: Add 1ml Extraction Buffer to dissolve before use. Then add 0.1ml this solution to 0.9 ml Extraction Buffer and mix well. The concentration is 0.1 mg/ml, Aliquot and stored at -20°C for upto 1 month. Avoid repeated freeze-thaw cycles, prepare fresh working solution for each experiment.

Working Reagent: Prepare according to the ratio as Reagent I : Reagent II : Reagent III =10:1:1 before use.

Standard: Each vial of standard contains 10 mg of ammonium ferrous sulfate hexahydrate, add 2.55 ml of standard diluent to each vial to obtain 10 umol/ml ferrous stock solution. Aliquot the stock solution and store at -20C for upto 3 months prepare fresh working solution for each experiment

Standard Concentration	Standard Vial	Dilution Particulars
4 umol/ml	Standard No.6	80 ul Standard (10 umol/ml) + 120 ul Standard Diluent
2 umol/ml	Standard No.5	100 ul Standard No.6 + 100 ul Standard Diluent
1 umol/ml	Standard No.4	100 ul Standard No.5 + 100 ul Standard Diluent
0.5 umol/ml	Standard No.3	100 ul Standard No.4 + 100 ul Standard Diluent
0.25 umol/ml	Standard No.2	100 ul Standard No.3 + 100 ul Standard Diluent
0.125 umol/ml	Standard No.1	100 ul Standard No.2 + 100 ul Standard Diluent
0.625 umol/ml	Standard No.0	100 ul Standard No.1 + 100 ul Standard Diluent

Assay Procedure:

1. Preheat the microplate reader or Visible Spectrophotometer for more than 30 min, and adjust the wavelength to 593 nm, Visible Spectrophotometer deionized water zero.
2. Add the following reagents respectively into each well:

Reagent	Blank well (ul)	Standard well (ul)	Test well (ul)	Positive Control well (ul)
Standard Diluent	10	0	0	0
Standard solution	0	10	0	0
Sample	0	0	10	0
Positive Control	0	0	0	10
Working Reagent	190	190	190	190

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

Note for Assay Protocol:

1. **In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 2.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.**
2. **If the sample has a noticeable color, a Sample Control well containing 10 ul of sample plus 190 ul of Reagent I may be set up, and $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Sample Control}}$.**

Data analysis:

1. Drawing of standard curve:
With the concentration of the standard solution as the y-axis and the $\Delta A_{\text{Standard}}$ as the x-axis, draw the standard curve. Substitute the ΔA_{test} into the equation to obtain the y value (umol/ml).
2. Calculation TAC of samples:
Unit Definition: The TAC of the sample is defined as the standard solution Fe^{2+} concentration (umol/ml) required to achieve the same absorbance change value (ΔA).
 - i. Calculated by sample fresh weight

$$\text{TAC(umol/g fresh weight)} = y \times V_{\text{sample}} \div (V_{\text{sample}} \div V_{\text{sample Total}} \times W) \times n = y \div W \times n$$
 - ii. Calculated by protein concentration

$$\text{TAC(umol/mg prot)} = y \times V_{\text{sample}} \div (V_{\text{sample}} \times \text{Cpr}) \times n = y \div \text{Cpr} \times n$$
 - iii. Calculated by Cells or Bacteria number

$$\text{TAC(umol/10}^4 \text{ cell)} = y \times V_{\text{sample}} \div (V_{\text{sample}} \times V_{\text{sample Total}} \div \text{Cells or Bacteria number}) \times n = y \div \text{Cells or Bacteria number} \times n$$
 - iv. Calculated by liquid volume

$$\text{TAC(umol/ml)} = y \times V_{\text{sample}} \div V_{\text{sample}} \times n = y \times n$$

Where:

 V_{sample} : sample volume added, 0.01 ml; $V_{\text{sample Total}}$: Extraction Buffer volume added, 1ml;

W: sample weight, g;

n: dilution multiple of sample further dilution;

Cpr: sample protein concentration, mg/ml;

Cells or Bacteria number: Total number of bacteria or cells, 10^4 as the unit.**Safety Precautions:**

- **This kit is For Research Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



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