Antioxidant Assay Kit

Catalog Number KA1622

100 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

- Applications:

✓ Direct Assays: serum, plasma, urine, saliva and other biological samples, food and beverages.

✓ Drug Discovery/Pharmacology: effects of drugs on TAC.

- Features:

✓ Sensitive and accurate: Use 20 µL sample. Linear detection range from 1.5 to 1000 µM Trolox equivalents.

✓ Simple and high-throughput: The procedure involves addition of a single working reagent and incubation for 10 min. Can be readily automated as a high-throughput assay for thousands of samples per day.

Principle of the Assay

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.

Simple, direct and high-throughput assays for total antioxidant capacity (TAC) find wide applications in research, food industry and drug discovery. Antioxidant Assay Kit measures total antioxidant capacity in which Cu^{2+} is reduced by antioxidant to Cu^{+}. The resulting Cu^{+} specifically forms a colored complex with a dye reagent. The color intensity at 570nm is proportional to TAC in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Reagent A	12 mL
Reagent B	1 mL
Standard: 50 mM Trolox	100 µL

Storage Instruction

Store Reagent A at room temperature and other components at -20℃. Shelf life of six months after receipt.

Materials Required but Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, plate reader capable of reading optical density at 570nm, homogenizer or sonicator etc.

Precautions for Use

- Precautions

✓ Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Sample Preparation

Samples should not contain any metal chelators (e.g. EDTA) and should be clear and free of any turbidity or particles. Liquid samples (e.g. non-hemolyzed serum, plasma) can be assayed directly. Cell lysate is prepared by homogenizing or sonicating cells in ice-cold 1 x PBS and centrifugation for 10 min at 14,000 rpm to pellet any debris. Use the clear supernatant for the assay. If not assayed immediately, freeze supernatant at -80℃ (stable for 1 month).

Assay Procedure

1. Standards and Samples. Equilibrate all components to room temperature. Briefly centrifuge Reagent B and Standard before opening. Mix 5 µL of the standard with 245 µL dH_2O (final 1 mM Trolox). Dilute standards as shown in the Table below. Transfer 20 µL standards into wells of a clear flat-bottom 96-well plate.

No	1 mM Trolox + H_2O	Vol (µL)	Trolox (µM)
1	100µL + 0µL	100	1000
2	60µL + 40µL	100	600
3	30µL + 70µL	100	300
4	0µL + 100µL	100	0

   Transfer 20 µL of each sample into separate wells of the 96-well plate.

   Note: for unknown samples, perform several dilutions to ensure that TAC is within the linear range of 1.5 to 1000 µM Trolox equivalents.
2. Assay. Prepare enough Working Reagent for Sample and Standard wells by mixing, for each assay well, 100 µL Reagent A and 8 µL Reagent B. Add 100 µL Working Reagent to all assay wells. Tap plate to mix. Incubate 10 min at room temperature.
3. Read OD_{570nm} on a plate reader.

Note: if calculated TAC is higher than 1000 µM Trolox equivalents, dilute sample in dH_2O and repeat assay. Multiply the results by the dilution factor.

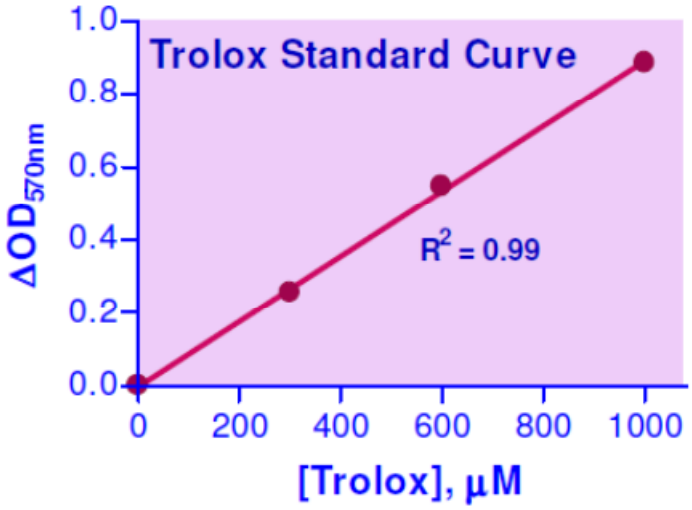
Data Analysis

Calculation of Results

Subtract blank OD value (#4) from all standard and sample OD values. Plot the ΔOD_{570nm} against standard concentrations and determine the slope of the standard curve. Calculate the Total Antioxidant Capacity (TAC) of Sample,

$$TAC = \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope\ (\mu M^{-1})} \times n\ (\mu M\ Trolox\ Equivalents)$$

OD_{SAMPLE} and OD_{BLANK} are the OD_{570nm} values of the sample and H_2O blank (standard #4). n is the sample dilution factor.



Resources

References

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