3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Antioxidant Assay Kit

Catalog Number **MAK334**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants protect cells from damages by reactive oxygen species which are produced by oxidation reactions within the cell. Antioxidants can be small molecules such as glutathione and vitamins, or macromolecules such as catalase and 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. The Antioxidant Assay Kit measures total antioxidant capacity in which Cu^{2+} is reduced by an antioxidant to Cu^{+}. The resulting Cu^{+} specifically forms a colored complex with a dye reagent. The color intensity at 570 nm is proportional to TAC in the sample. The kit uses 20 µL of sample and has a linear detection range from 1.5–1,000 µM Trolox equivalents.

This kit is suitable for antioxidant determination in serum, plasma, urine, saliva, and other biological samples, food, and beverages. The kit can also be utilized in drug discovery/pharmacology for studying the effects of drugs on TAC.

Components

The kit is sufficient for 100 colorimetric assays in96 well plates.

Reagent A Catalog Number MAK334A	12 mL
Reagent B Catalog Number MAK334B	1 mL
Standard (50 mM Trolox) µLCatalog Number MAK334C	100

Reagents and Equipment Required but NotProvided.

- Pipetting devices and accessories(e.g., multichannel pipettor)
- Centrifuge tubes
- 96 well flat bottom plate. It is recommended to useclear plates for colorimetric assays
- Spectrophotometric multiwell plate reader
- Homogenizer or sonicator
- Phosphate buffered saline (PBS)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safehandling practices.

Storage/Stability

The kit is shipped at room temperature. Store allcomponents at –20 °C upon receiving.

Preparation Instructions

Reagent Preparation

Equilibrate all components to room temperature. Briefly centrifuge Reagent B and Standard before opening. Reagent B may appear as an emulsion upon thawing. Reagent B can be warmed at 37°C to return it to a clear solution without affecting performance.

Reaction Mix Preparation
Prepare enough Reaction Mix for Sample and Standardwells by mixing, for each assay well: 100 µL of Reagent A and 8 µL of Reagent B.2

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× PBS and centrifugation for 10 minutes at 14,000 rpm to pellet any debris. Use the clear supernatant for the assay. If not assayed immediately, freeze supernatant at –80 °C (stable for 1 month).

Procedure
Trolox Standards
Prepare 250 µL of a 1 mM Trolox Standard by combining 5 µL of Standard with 245 µL of ultrapure water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table 1.

Table 1.
Preparation of Trolox Standards

Tube	1 mM Premix	Ultrapure Water	Trolox (µM)
1	100 µL	0 µL	1,000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	0 µL	100 µL	0

Assay Reaction
1. Transfer 20 µL of standards into separate wells of a clear, flat bottom 96 well plate.
2. Transfer 20 µL of samples into separate wells of a clear, flat bottom 96 well plate.
   Note: For unknown samples, perform several dilutions to ensure that TAC is within the linear range of 1.5–1,000 µM Trolox equivalents.
3. Add 100 µL of Reaction Mix to all assay wells.
4. Tap plate to mix and incubate for 10 minutes at room temperature.
5. Measure the absorbance at 570 nm (A_{570}).

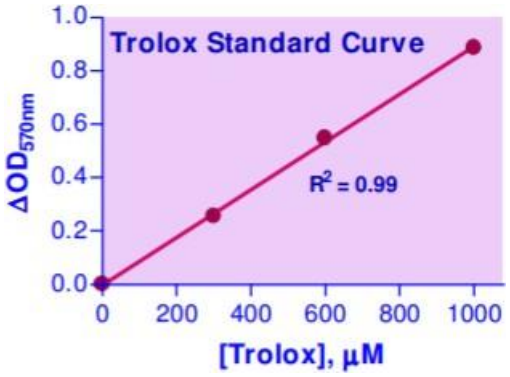
Results
Subtract blank A_{570} value (Standard Tube 4) from all standard and sample A_{570} values. Plot the ΔA_{570} against standard concentrations and determine the slope of the standard curve. Calculate the Total Antioxidant Capacity (TAC) of Sample as follows:

$$TAC\ (\mu M) = \frac{(A_{570})_{sample} - (A_{570})_{blank}}{Slope\ (\mu M^{-1})} \times n$$

where:
$(A_{570})_{sample}$ = the absorbance of the sample
$(A_{570})_{blank}$ = the absorbance of the medium blank (Standard Tube 4)
n = sample dilution factor

Note: If calculated TAC is higher than 1,000 µM Trolox equivalents, dilute sample in ultrapure water and repeat assay. Multiply the results by the dilution factor.

Figure 1.
Typical Standard Curve



References

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