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Product Information

Total Antioxidant Capacity Assay Kit

Catalog Number **MAK187**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Oxidants, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), can generate free radicals that can cause severe oxidative damage to cellular lipids, membranes, proteins, and DNA.[1] Antioxidants can scavenge these free radicals and prevent cellular oxidative stress by enzymatic and non-enzymatic mechanisms. Enzyme systems that function as antioxidants include catalase and peroxidase. Tocopherols, carotenes, vitamin A, and ubiquinols function as lipid-soluble antioxidants; whereas, glutathione and ascorbate are some of the water-soluble antioxidants.[2] Measurement of the total non-enzymatic antioxidant capacity (TAC) of biological samples is indicative of their ability to counteract oxidative stress-induced damage in cells. TAC is used to provide insights into the development and treatment of oxidative-stress related disorders.

In the Total Antioxidant Capacity Assay Kit, either the concentration of the combination of both small molecule and protein antioxidants, or the concentration of only small molecule antioxidants can be determined. Cu^{2+} ion is converted to Cu^{+} by both small molecules and proteins. However, the use of the Protein Mask prevents Cu^{2+} reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu^{+} ion chelates with a colorimetric probe, giving a broad absorbance peak at ~570 nm, which is proportional to the total antioxidant capacity. The kit gives antioxidant capacity in Trolox equivalents (ranging from 4–20 nmole/well). Trolox, a water-soluble vitamin E analog, serves as an antioxidant standard.

Components

The kit is sufficient for 100 assays in 96 well plates.

Cu^{2+} Reagent 0.2 mL
Catalog Number MAK187A

Assay Diluent 10 mL
Catalog Number MAK187B

Protein Mask 10 mL
Catalog Number MAK187C

Trolox Standard, 1 μmole 1 vl
Catalog Number MAK187D

Reagents and Equipment Required but Not Provided.

- DMSO (dimethyl sulfoxide Catalog Number D2650 or equivalent)
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

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 safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents, samples, and standards. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Cu^{2+} Reagent – Allow to come to room temperature before use. Dilute one part of the Cu^{2+} Reagent with 49 parts of Assay Diluent to prepare Cu^{2+} Working Solution.

Assay Diluent and Protein Mask – Allow reagents to come to room temperature before use.

Trolox Standard – Reconstitute with 20 μL of DMSO and vortex. Add 980 μL of water to generate 1 mM Trolox Standard Solution. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 4 months.

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Trolox Standards for Colorimetric Detection
Add 0, 4, 8, 12, 16, and 20 μL of the 1 mM (1 nmole/μL) Trolox Standard Solution into a 96 well plate, generating 0 (blank), 4, 8, 12, 16, and 20 nmole/well standards. Add water to each well to bring the volume to 100 μL.

Sample Preparation
Serum, urine, and culture media samples can be directly added to the wells.

For serum samples, it is suggested to assay 0.01–0.1 μL without Protein Mask or 1–10 μL with Protein Mask.

For small molecule TAC, dilute samples at 1:1 ratio with Protein Mask.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Add 1–100 μL of sample into wells. Bring samples to a final volume of 100 μL with water.

Assay Reaction
1. Add 100 μL of Cu^{2+} Working Solution to all standard and sample wells.

2. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 90 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 570 nm (A_{570}).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Trolox Standard. Correct for the background by subtracting the 0 (blank) standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Trolox Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Using the corrected measurement, Trolox equivalents of the sample may be determined from the standard curve.

Concentration of Trolox Equivalents

$S_a/S_v = C$

where:

S_a = Trolox equivalent of unknown sample well (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of antioxidant in sample (nmole/μL or mM Trolox equivalents)

Sample Calculation

Amount of antioxidant = 4.84 nmole (from Trolox standard curve)

Sample volume (S_v) = 50 μL

Concentration of antioxidant (Trolox equivalents) in sample:

4.84 nmole/50 μL = 0.0968 nmole/μL

References

1. Valko, M. et al., Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., **39(1)**, 44–84 (2006).
2. Sies, H., Oxidative stress: oxidants and antioxidants. Exp. Physiol., **82(2)**, 291–295 (1997).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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