

Fully Automated 3rd Generation Total Antioxidant Status (TAS) ASSAY KIT

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Summary and Explanation

Reactive oxygen species (ROS) is produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms that remove them via enzymatic and non-enzymatic antioxidative mechanisms. Under certain conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidative or in over 100 disorders, develops¹.

Antioxidant molecules prevent or inhibit these harmful reactions². Serum (or plasma) concentrations of different antioxidants can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive, costly, and require complicated techniques. Because the measurement of different antioxidant molecules separately is not practical and their antioxidant effects are additive, the total antioxidant capacity of a sample is measured, and this is called total antioxidant capacity (TAC)³, total antioxidant activity (TAA)⁴, total antioxidant power (TAOP)⁵, total antioxidant status (TAS)⁶, total antioxidant response⁷, or other synonyms.

Principle of Assay

Antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analog.

Components

All reagents and standards are ready to use.

- **Reagent 1** (Assay Buffer) 1 x 50 ml
- **Reagent 2** (Colored ABTS Radical Solution) 1 x 10 ml
- ***Standard 1** (0.0 mmolTrolox Equiv./L) Solution(Not included)
- **Standard 2** (1.0 mmolTrolox Equiv./L) Solution 1 x 10 ml

*You should use any deionised-water

Storage Conditions

This kit should be stored at 4°C.

Additional Items Required

A spectrophotometer or a plate reader or an automated biochemistry analyzer.

1. Manual Study

- Place 500 microliter Reagent 1 in cell and add 30 microliter standard (or sample). Read the initial absorbance at 660 nm for the first absorbance point.
- Add 75 microliter Reagent 2 to the cell and incubate 10 min at room temperature or 5 min at 37°C. Read the absorbance a second time at 660 nm.

Calculating the Results

$$\text{Result} = \frac{[(\Delta\text{Abs Std1}) - (\Delta\text{Abs Sample})]}{[(\Delta\text{Abs Std1}) - (\Delta\text{Abs Std2})]}$$

Δ Absorbance Standard1= (Second Absorbance of Std1- First Absorbance of Std1)

Δ Absorbance Standard2 = (Second Absorbance of Std2- First Absorbance of Std2)

Δ Sample Absorbance = (Second Absorbance of Sample- First Absorbance of Sample)

2. **Automated measurement** is performed as same procedure. Only incubation time is shortened from 10 min to 5 min. Other parameters are similar. The volumes of reagents and sample are reduced at same ratio.

References

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4. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001;54(5):356-61.
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