RelAssay Diagnostics®

Clinical Chemistry Solutions

Fully Automated

Total Oxidant Status (TOS)

ASSAY KIT

Product Code: RL0024

MANUFACTURER :

Mega Tıp San ve Tic Ltd Sti
Bahcelievler Mah K.Ismail Pasa Sk No: 10 Sahinbey/Gaziantep/TURKEY
Phone: +90 342 230 37 52

www.relassay.com
support@relassay.com

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Total Oxidant Status (TOS) Assay Kit

Summary and Explanation

Reactive oxygen and nitrogen species are 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, develops[1].

Serum (or plasma) concentrations of different oxidant species can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive and costly and require complicated techniques[2]. Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the total oxidant status (TOS) of a sample is measured and this is named total peroxide (TP)[1,3,4,5], serum oxidation activity (SOA)[6], reactive oxygen metabolites (ROM)[7] or some other synonyms.

Principle of Assay

Oxidants present in the sample oxidize the ferrous ion–chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L)[8].

Components

All reagents are ready to use.

- **Reagent (1Assay Buffer) : 50 ml X 1**
- **Reagent 2 (Prochromogen solution) : 10 ml X 1**
- **Standard 1 (Blank Solution)* (Not included)**
- **Standard 2 Solution** : 10 ml X 1**

*****Use any deionised-water (0.0 μmol H_2O_2 Equiv./L) [*This solution is used to check kit quality and not used for calibration processes. If the obtained absorbance using this solution is greater than 0.500, discharge this kit please*].

******[Stock Stabilized Standard Solution (SSSS)] (800 mM H_2O_2Equiv./L)

Storage Conditions

This kit should be stored at 2-8°C.

Additional Items Required

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

Samples

Blood serum, heparinised plasma, semen plasma, saliva, urine, cell lysates and tissue homogenates can be used as sample.

Procedure

1. Manual Study. Prepare working standard solution. SSSS is diluted 40,000 times with deionised water. A liquid of 50 microliter SSSS is added to 10 ml deionised water and vortexed (The first step dilution). A liquid of 50 microliter of the prepared solution is added to 10 ml deionised water and vortexed (The second step dilution). The final concentration of the working standard is **20 micromolar H_2O_2**. Prepare working solution daily.

- Place 500 microliter Reagent 1 in cell and add 75 microliter the prepared standard (or sample). Read the initial absorbance at 530 nm for the first absorbance point.
- Add 25 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 530 nm.

Calculating the Results

Result = **(ΔAbsSample / ΔAbsStandard2) X 20 (Standard2 Value)**

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

Δ Absorbance Standard 2 = (Second Absorbance of Std 2 − First Absorbance of Std 2)

Standart 2 Value = 20 μmol H_2O_2 Equiv./L

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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