Total Oxidant Status (TOS) Assay Kit

Quantitative Colorimetric Determination of Total Oxidant Status

DESCRIPTION

Under acidic conditions, the oxidizing material in the sample can oxidize substances to produce a blue-purple complex. When the pH of the solution is in the range of 2-3, its maximum absorption wavelength is around 545 nm, and the color intensity is proportional to the content of oxidation substances in a certain concentration and at a certain time, so the total oxidation state of the sample can be indirectly calculated. TOS may contribute to the pathology of many diseases including atherosclerosis, diabetes, and Alzheimer's. Simple, direct, and high-throughput assays for TOS find wide applications in research, Physiology, Nutrition, Botany, Food industry, environmental sciences, reproduction, and drug discovery.

KEY FEATURES

Sensitive and accurate. 2.5 µmol H_2O_2 Equiv./L
Simple and high-throughput. The procedure involves the addition of a single working reagent that can be readily automated as a high-throughput assay for thousands of samples per day.

APPLICATIONS

Direct Assays: serum, plasma, urine, saliva and other biological samples, food and beverages.
Drug Discovery/Pharmacology: effects of drugs on TOS

KIT CONTENTS 100 Reaction Kit

R1-50 ml R2- 3 ml
Standard: 1 mL H_2O_2
Prepare 25 µmol /L H_2O_2. Take 1 ml of H2O2 and dilute it with 10 ml distil H_2O. After that take 12.15 µl into 50 ml to prepare 25 µmol/L.

No	25 µmol /L H_2O_2 + Distilled water	Vol (µL)
1	500 µL + 500 µL	1000
2	250 µL + 500 µL	750
3	125 µL + 500µL	625
4	75 µL + 500 µL	575
5	37.5 +500 µL	537.5
6	0+ 500 µL	500

Storage conditions. The kit is shipped at room temperature. Store all components at room temperature for 6 month. Shelf life can be increased if stored at -40°C for one year.

Precautions: reagents are for research use only. Normal precautions forlaboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION

Serum sample: Blood should be collected in a gel tube, then centrifuged at 4000 rpm for 5 min, and obtained clear solution on top of the cellular material. Separate it into a new Eppendorf tube and use it for further processing.

Tissue homogenate: Cell lysate is prepared by homogenizing tissue (25mg) or sonicating cells in ice-cold 1 x PBS (1mL) 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°C (stable for 1 month).

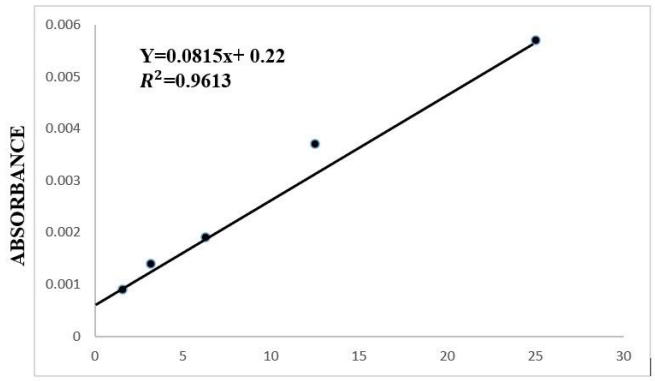
Sr.	Reagents	Volume
1	Sample	70 µL
2	R1	450 µL
3	R2	22 µL

ASSAY PROCEDURE

1. To begin, segregate Eppendorf tubes on the based total number of samples.
2. Subsequently, in each Eppendorf tube, systematically introduce the following components one after the other.
3. Following the addition of the specified components, all Eppendorf tubes were incubated for 4 minutes in the dark.
4. Read OD545nm on a plate reader.
5. Note: If the calculated TOS is higher than 1000 µM H_2O_2 equivalents, dilute the sample in dH2O and repeat the assay. Multiply the results by the dilution factor.

CALCULATION

Subtract blank OD value from all standard and sample OD values. Plot the OD 545nm against standard concentrations and determine the slope of the standard curve. Calculate the Total Oxidant Status (TOS) of the Sample,



MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, plate reader capable of reading optical density at 545 nm, homogenizer or sonicator etc.

PUBLICATIONS

1. Saleem, S., Mukhtar, I., Aati, H. Y., Muzaffar, H., Anwar, H., Hussain, M., Ahmad, M., & Umair, M. (2024). Effects of Withania somnifera (L.) Dunal in acute pulmonary pathophysiology in a rat model of smoke-induced lung injury and role of IRS-1 and SOX-2. *South African Journal of Botany*, *171*, 757-767.