Total Oxidant Status Assay Kit

Cat. No. kit-2173 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	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 H2O2 Equiv./L).
Storage	2-8°C
Kit 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 H2O2 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 H2O2Equiv./L)
Materials Required but Not Supplied	A spectrophotometer or a plate reader or an automated biochemistry analyzer.

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Compatible Sample Types	Blood serum, heparinised plasma, semen plasma, saliva, urine, cell lysates and tissue homogenates can be used as sample.
Assay Protocol	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 37oC. Read the absorbance a second time at 530 nm. Calculating the Results Result = (ΔAbsSample /ΔAbsStandard2) X 20 (Standard2 Value) ΔSample Absorbance = (Second Absorbance of Sample - First Absorbance of Sample) ΔAbsorbance 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.

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