Total Oxidant Status (TOS) Assay Kit

Catalog No.: abx092300

Size: 96 tests

Detection Range: 2.5 µmol H_2O_2 Equivalent/L – 100 µmol H_2O_2 Equivalent/L

Sensitivity: 2.5 µmol H_2O_2 Equivalent/L

Storage: Store all components at 4°C. Store all components in the dark.

Application: For detection and quantification of Total Oxidant Status in serum, plasma, tissue homogenates, cell lysates, and other biological fluids.

Assay Principle

The Total Oxidant Status refers generally to the amount of oxidizing material in a given sample. The majority of the oxidizing chemicals in living organisms will be reactive oxygen species (ROS), which contribute to a large number of different pathologies, such as cancer from direct DNA damage, heart disease, and diseases of the nervous system. However, many other chemicals can also contribute to Total Oxidant Status, making it useful as a measure of general health. As such, the ability to quantify and compare the Total Oxidant Status of samples is of interest to researchers in numerous different fields – especially those investigating conditions with complex pathologies.

Abbexa's Total Oxidant Status (TOS) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating the Total Oxidant Status of a sample. Under acidic conditions, oxidizing material in the sample can oxidize Fe^{2+} ions to Fe^{3+}. Ferric iron binds strongly with the chromogen xylenol orange, producing a blue-purple complex with an absorbance maximum of 590 nm under acidic conditions (pH 2 – 3). The intensity of the color is proportional to the Total Oxidant Status of the sample, which can then be calculated.

Kit components

1. 96-well microplate
2. Chromogenic Reagent: 24 ml
3. Substrate: 6 ml
4. Standard (200 µmol/L): 1 ml
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (590 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Centrifuge
6. Vortex mixer
7. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh out at least 20 mg of tissue, and wash in ice-cold PBS (0.01 M, pH 7.4). Add the tissue into ice-cold PBS in a ratio of 1 : 9 weight (mg) to volume (µl) (e.g. for 20 mg of tissue, add into 180 µl ice-cold PBS). Homogenize manually, using a mechanical homogenizer or by ultrasonication, at 4°C. Collect the tissue homogenate, and centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant and assay immediately, or aliquot and store at -80°C for up to 1 month. Keep on ice until detection.

  Note: To calculate Total Oxidant Status in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.
- **Cell Lysates:** Collect at least 1 × 10^6 cells, and wash in ice-cold PBS (0.01 M, pH 7.4). Suspend the cells in ice-cold PBS in a ratio of 1 × 10^6 cells : 200 µl ice-cold PBS (e.g. for 1 × 10^6 cells, add into 200 µl ice-cold PBS). Homogenize manually by ultrasonication at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant and assay immediately, or aliquot and store at -80°C for up to 1 month. Keep on ice until detection.

  Note: To calculate Total Oxidant Status in cell lysates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double-distilled water, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Pig serum	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat lung tissue homogenate	1
0.612×10^6 Human MOLT-4 cells	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Standards:** Label 7 tubes with 100 µmol/L, 80 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, 20 µmol/L, and 10 µmol/L. Add 100 µl, 80 µl, 60 µl, 50 µl, 40 µl, 20 µl, and 10 µl of Standard to the 100 µmol/L, 80 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, 20 µmol/L, and 10 µmol/L tubes respectively, followed by 100 µl, 120 µl, 140 µl, 150 µl, 160 µl, 180 µl, and 190 µl of double-distilled water, to prepare Standard Dilutions with concentrations 100 µmol/L, 80 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, 20 µmol/L, and 10 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**100**	**80**	**60**	**50**	**40**	**20**	**10**
Standard (µl)	100	80	60	50	40	20	10
Double-distilled water (µl)	100	120	140	150	160	180	190

For the blank, or 0 µmol/L standard, use pure double-distilled water. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- The Chromogenic Reagent is highly sensitive to contamination. It is recommended to aliquot the Chromogenic Reagent into smaller quantities before use, and avoid working directly out of the Chromogenic Reagent vial.
- The Substrate is sensitive to air. Do not leave the vial open for long periods, and seal as soon as possible after opening.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

Plate the samples slowly to avoid bubbles. Ensure there are no bubbles present during reading.

1. Mark positions on the microplate for each standard and sample. *It is strongly recommended to prepare all wells in duplicate.*
2. Add 20 µl of each standard dilution to the corresponding standard wells.
3. Add 20 µl of each sample to the sample wells.
4. Add 200 µl of Chromogenic Reagent to all wells.
5. Tap or shake the plate with a microplate shaker to mix fully for 5 seconds.
6. Measure the OD of each well with a microplate reader at 590 nm. For each well, record this reading as “OD 1”

7. Add 50 µl of Substrate to each well.
8. Cover the plate with a plate sealer, and then tap or shake the plate with a microplate shaker to mix fully for 5 seconds.
9. Incubate at 37°C for 5 minutes.
10. Remove the plate sealer and measure the OD of each well with a microplate reader at 590 nm. For each well, record this reading as “OD 2”.
11. For each well, calculate the difference between OD 2 and OD 1 ($\mathrm{OD}_2 - \mathrm{OD}_1$). Record this value as “ΔOD”.

C. Calculation of Results

Plot the standard curve, using the ΔOD of the standard dilutions (adjusted for the ΔOD of the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the Total Oxidant Status in each sample well can be derived with the following formulae:

1. Liquid samples:

$$\text{Total Oxidant Status (µmol } H_2O_2 \text{ Equiv./L)} = F \times \frac{(\Delta OD_{Sample} - \Delta OD_{Blank} - b)}{a}$$

2. Tissue and **cell samples:**

$$\text{Total Oxidant Status (µmol } H_2O_2 \text{ Equiv./gprot)} = F \times \frac{(\Delta OD_{Sample} - \Delta OD_{Blank} - b)}{a \times C_{Protein}}$$

where:

ΔOD_{Sample}	The ΔOD value of the sample wells
ΔOD_{Blank}	The ΔOD value of the blank wells
$V_{Substrate}$	Volume of sample + Substrate (0.15 ml)
$C_{Protein}$	Concentration of protein in sample (mg/ml)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.