Total Antioxidant Status Assay Kit

Catalog No.: abx295018

Size: 96 tests

Detection Range: 0.23 mmol Trolox Equiv./L - 2 mmol Trolox Equiv./L

Sensitivity: 0.23 mmol Trolox Equiv./L

Storage: Store all components in the dark at -20°C for up to 6 months.

Application: For determination of total antioxidant status (TAS) in serum, plasma, urine, cell culture supernatants, and animal and plant tissue homogenates.

Introduction

Reactive oxygen species (ROS) in aerobic organisms are highly reactive products continuously formed in metabolism of oxygen. ROS are essential in many cellular processes; however, they can also cause oxidative damage. It is therefore important that ROS production and elimination is controlled. ROS elimination is controlled by antioxidants, which can be enzymatic or non-enzymatic. The total antioxidant status of a system is reflected by the total level of various antioxidant macromolecules, small molecules and enzymes.

ABTS is oxidized to green $ABTS^{\bullet+}$ by oxidants, which can be reduced back to colorless ABTS in the presence of antioxidants. The Total Antioxidant Status (TAS) of the sample can be determined and calculated by measuring the absorbance of $ABTS^{\bullet+}$ at 660 nm. Trolox, an analog of Vitamin E which also has a similar antioxidant state, is used as a reference substance for total antioxidant status.

Kit components

1. 96-well microplate
2. Buffer Solution: 30 ml
3. Chromogenic Reagent: 5 ml
4. Standard (2 mmol/L): 2 × 2 ml
5. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (650-670nm)
2. Double-distilled water
3. 60% ethanol
4. Micropipette and pipette tips
5. Microcentrifuge tubes
6. 37°C incubator

Protocol

A. Preparation of samples

Bring all reagents to room temperature before use.

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:** Liquid samples such as serum and plasma should be collected using conventional methods and can be used directly in this assay.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml of 60% ethanol. Homogenize by hand using a mechanical homogenizer in an ice bath. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT or 2-merhydryl ethanol.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples with 60% ethanol. The suggested starting dilution factor for various sample types is below:

Sample Type	Dilution Factor
Mouse Liver Tissue Homogenate (10%)	1
Rat Liver Tissue Homogenate (10%)	1
Rat Lung Tissue Homogenate (10%)	1
MOLT4 Cell Supernatant	1
Human Urine	8-10
Human Serum	1
Human Saliva	1
Mouse Serum	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.

B. Assay Procedure

1. **Standard curve preparation:** Label 7 tubes with 2, 1.8, 1.6, 1.4, 1.2, 0.8, 0.4 and 0 mmol/L. Dilute 2 mmol/L of standard solution with 60% ethanol to concentrations of 2, 1.8, 1.6, 1.4, 1.2, 0.8 and 0.4 mmol/L. The 60% ethanol serves as the 0 mmol/L (blank) standard.

2. Sample Measurement:

2.1. Set the Standard and Sample wells on the well-plate.
2.2. Add 10 µl of prepared standard to the standard wells.
2.3. Add 10 µl of sample to the sample wells.
2.4. Add 200 µl of Buffer Solution to each well.
2.5. Measure the OD values of each well at 660 nm with a microplate reader and record the value as A_1.
2.6. Add 20 µl of Chromogenic Reagent to each well. Mix by pipetting up and down 5-6 times.
2.7. Incubate at 37°C for 5 min.
2.8. Measure the OD values of each well at 660 nm with a microplate reader and record the value as A_2.
2.9. Calculate $\Delta A = A_2 - A_1$.

C. Calculation of Results

The standard curve can be plotted as the blank-corrected OD value of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = \mathrm{a}x + \mathrm{b}$). Blank correction is recommended ($y = \Delta A_{\mathrm{Standard}} - \Delta A_{\mathrm{Blank}}$). Trolox is used as a reference substance for total antioxidant status.

1. Liquid samples:

$$\mathrm{TAS\ (mmol\ Trolox\ Equiv./L)} = \frac{(\Delta A_{\mathrm{Blank}} - \Delta A_{\mathrm{Sample}} - \mathrm{b})}{\mathrm{a}} \times f$$

2. Tissue and cell lysate samples:

$$\mathrm{TAS\ (mmol\ Trolox\ Equiv./kg\ wet\ weight)} = \frac{(\Delta A_{\mathrm{Blank}} - \Delta A_{\mathrm{Sample}} - \mathrm{b})}{\mathrm{a}} \times \frac{V}{m} \times f$$

where:

ΔA	$A_2 - A_1$
$\Delta A_{\mathrm{Blank}}$	ΔA of the 0 mmol/L standard (blank)
$\Delta A_{\mathrm{Sample}}$	ΔA of the sample
y	blank-corrected OD of the standard ($\Delta A_{\mathrm{Standard}} - \Delta A_{\mathrm{Blank}}$)
x	concentration of the standard (mmol/L)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
V	volume of tissue homogenate sample (ml)
m	weight of tissue homogenate sample (g)