Total Antioxidant Capacity Assay Kit (Colorimetric Method)

Catalog No.: abx090678

Size: 100 tests (50 samples)

Range: 0.2 U/ml - 55.2 U/ml

Detection Limit: 0.2 U/ml

Storage: Store at 2-8 °C in the dark for up to six months.

Application: For detection the TAC in serum, plasma, whole blood, and animal or plant tissue homogenates.

Introduction

Total antioxidant capacity (TAC) determinations are simple, inexpensive, and able to evaluate the capacity of known and unknown antioxidants and their additive, synergistic and/or antagonistic actions, in chemical and biological systems. However, different TAC assays correlate poorly with each other; since each TAC assay is sensitive to a particular combination of compounds, but exclude many others. The TAC values for foods cannot be translated to the in vivo (human) antioxidant defenses, and furthermore, to health effects provided by that food. Alcoholism causes an impaired antioxidant capacity and a decreased secretion of amylase, which is ameliorated due to the alcohol withdrawal regimen. The strong correlation between blood and saliva with respect to the antioxidants suggests the potential future use of saliva as a laboratory tool in clinical medicine.

Kit Components

Reagent	Format	Size
Reagent A	Liquid	2 × 60 ml
Reagent B	Lyophilized	2 vials
Reagent C	Liquid	5 ml
Reagent C diluent buffer	Liquid	60 ml
Reagent D	Liquid	24 ml
Reagent E	Liquid	24 ml

Material Required But Not Provided

1. Microplate reader or spectrophotometer (wavelength: 520 nm)
2. 37 °C incubator/water bath
3. Pipette and disposable pipette tips
4. Centrifuge
5. Vortex mixer

Protocol

Reagent Preparation

- **Reagent B working solution:** Reconstitute each vial of Reagent B with 120 ml of double-distilled water. Incubate at 37 °C in a water bath to fully dissolve the reagent before use.
- **Reagent C working solution:** Dilute Reagent C with Reagent C diluent buffer 1/19-fold to make the Reagent C working solution (i.e. add 1 ml of Reagent C into 18 ml of Reagent C diluent buffer to prepare 19 ml of Reagent C working solution).
- **Reagent E:** Incubate Reagent E at 37 °C in a water bath until the reagent is a transparent liquid.
- **Mix reagent:** Add Reagent A, Reagent B working solution and Reagent C working solution together in the following ratio: 2:4:1 (i.e. add 2 ml Reagent A, 4 ml Reagent B working solution and 1 ml Reagent C working solution to prepare 7 ml of Mix reagent).

Sample Preparation

- **Serum and plasma:** Bring to room temperature before use. These samples can be analyzed directly.
- **Whole blood:** Dilute samples with double distilled water to a ratio of 1:9 (i.e. add 1 ml of whole blood into 9 ml of double distilled water).
- **Tissue homogenates:** Cut samples and prepare with saline solution. Centrifuge samples at 2000-3000 RPM for 20 min to homogenize thoroughly. Collect the supernatant.

Assay Procedure – Serum, Plasma and Whole Blood

1. Set the Test and Control vials.
2. Add 0.1 ml of serum or plasma; or 0.05 ml of diluted whole blood to each Test vial.
3. Add 3.5 ml of Mix reagent to each vial.
4. Vortex each vial to mix thoroughly, then place in a water bath set to 37 °C for 30 min.
5. Add 0.1 ml of Reagent D to each vial.
6. Add 0.1 ml of serum or plasma; or 0.05 ml of diluted whole blood to each Control vial.
7. Vortex each vial to mix thoroughly, and allow to stand at room temperature for 10 min.
8. Read the O.D. absorbance of each vial at 520 nm, 1 cm optical path in a spectrophotometer.

Assay Procedure – Tissue Homogenates

1. Set the Test and Control vials.
2. Add 0.1-0.2 ml of tissue homogenate sample to each Test vial.
3. Add 3.5 ml of Mix reagent to each vial.

4. Vortex each vial to mix thoroughly, then place in a water bath set to 37 °C for 30 min.
5. Add 0.2 ml of Reagent D to each vial.
6. Add 0.1-0.2 ml of sample to each Control vial.
7. Add 0.2 ml of Reagent E to each vial.
8. Vortex each vial to mix thoroughly, and allow to stand at room temperature for 10 min.
9. Read the O.D. absorbance of each vial at 520 nm, 1 cm optical path in a spectrophotometer.

Calculations

1. Serum, plasma and whole blood

$$\textbf{TAC Activity}\ (\mathbf{U/ml}) = \frac{(\mathbf{OD_{Vial}} - \mathbf{OD_{Control}}) \times \mathbf{V_{Total}}}{\mathbf{0.01} \times \mathbf{T} \times \mathbf{V_{Sample}}} \times \mathbf{DF}$$

2. Tissue homogenates

$$\textbf{TAC Activity}\ (\mathbf{U/mg\ protein}) = \frac{(\mathbf{OD_{Vial}} - \mathbf{OD_{Control}}) \times \mathbf{V_{Total}}}{\mathbf{0.01} \times \mathbf{T} \times \mathbf{V_{Sample}} \times \mathbf{C_{Protein}}} \times \mathbf{DF}$$

where

$\mathbf{OD_{Control}}$	the average optical density (at 520 nm) of the control vials
$\mathbf{OD_{Vial}}$	the optical density (at 520 nm) of the vial
$\mathbf{T}$	reaction time (30 minutes)
$\mathbf{V_{Total}}$	total volume of all reagents (ml, 3.7 ml for Serum/Plasma, 3.65 ml for Whole Blood)
$\mathbf{V_{Sample}}$	loading sample volume (ml)
$\mathbf{C_{Protein}}$	concentration of protein in tissue homogenate samples (mg protein/ml)
$\mathbf{DF}$	dilution factor of test samples