Total Antioxidant Capacity (TAC) Assay
Cat. No. 8168, 100 tests

Introduction

Antioxidants are a group of free radical scavengers, including macro and micro molecules (transferrin, vitamin E, etc.) and enzymes (catalase, GSH reductase, etc.), that can protect vital cellular target against oxidative attack from free radicals or reactive oxygen species (ROS). Due to the chemical diversity and cooperative nature of antioxidants, the measurement of total antioxidant capacity (TAC) is important in monitoring the clinical status of body fluids, as well as the cumulative capability of various food supplements to counteract oxidative stress. The ScienCell™ Total Antioxidant Capacity (TAC) Assay is a cupric ion reducing antioxidant capacity (CUPRAC) spectrophotometric method, which can simultaneously measure hydrophilic and lipophilic antioxidants at physiological pH. Trolox®, a cell-permeable, water-soluble derivative of vitamin E with antioxidant properties, serves as a standard.

Kit Components

Cat. No.	# of vials	Reagent	Amount	Storage
8168a	1	CUPRAC Reagent	30 ml	2-8ºC
8168b	1	TAC Assay Buffer	12.5 ml	2-8ºC
8168c	1	Trolox® Standard (0.5 mM)	2.5 ml	-20ºC

Quality Control

Data from ScienCell™ TAC Assay of Trolox® solutions with concentrations ranging form 0.0125 to 0.5 mM shows a linear relationship between OD_{450nm} and Trolox® concentration (Figure 1).

Procedures

A. Preparation of working standards:

1. Prepare a Trolox® standard curve using the serial dilutions of the 0.5 mM Trolox® standard according to Table 1. 350 µl of Trolox® solution is prepared for each point to provide three replicates of 110 µl.

B. Sample preparation:

1. Dry samples (both hydrophilic and lipophilic) should be appropriately solubilized with TAC Assay Buffer. Fluid samples should be measured directly after filtration and dilution with DI H_2O.

C. Assay procedure:

1. Add 110 µl of sample or standard solution into each well of 48-well plate.
2. Add 300 µl of CUPRAC Reagent into each well containing sample or standard; incubate for 30 minutes at room temperature.
3. Read absorbance at 450 nm using a plate reader.

D. Calculations:

1. Generate the standard curve by plotting the OD_{450nm} as a function of the Trolox® concentrations,

as shown in Figure 1.

No.	0.5 mM Trolox® (µL)	TAC Buffer (µL)	Trolox® (mM)
1	350	0	0.5
2	280	70	0.4
3	210	140	0.3
4	140	210	0.2
5	70	280	0.1
6	35	315	0.05
7	17.5	332.5	0.025
8	8.8	341.2	0.0125
9	0	350	0 (Blank)

Table 1. Preparation of Trolox® standards in TAC Assay.

2. Determine the antioxidant Trolox® equivalent concentration of each sample based on the Trolox® standard curve.

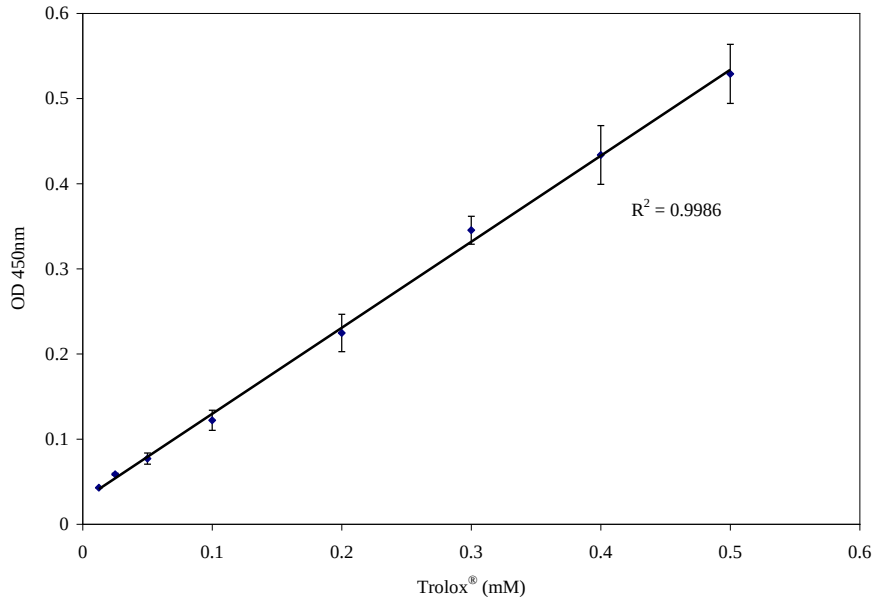

Figure 1 A typical Trolox® standard curve measured by ScienCell™ TAC Assay.