Cell Meter™ Colorimetric Antioxidant Activity Assay Kit

Catalog number: 15900
Unit size: 200 Tests

Component	Storage	Amount
Component A: Horseradish Peroxidase	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophylized powder)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: ReadiUse™ ABTS Substrate Solution	Freeze (<-15 °C), Minimize light exposure	1 bottle (35 mL)
Component D: Trolox	Refrigerate (2-8 °C), Minimize light exposure	1 vial (2.5 mg)

OVERVIEW

Reactive oxygen species (ROS) are important in cell signaling and maintaining physiological cell metabolism. Excessive level of ROS damages cellular components, which links to numerous diseases, including cancer, metabolic disorders, neurodegenerative disease etc. Antioxidants, including enzymes, macromolecules and small molecules are generated to combat the ROS induced damages in the living organisms. Quantitative analysis of antioxidant activity in body fluids, tissues and cells provides important biological information. Our Amplite™ Colorimetric antioxidant assay kit use ABTS an colorimetric indicator of antioxidant activity based on the observation that the ability of antioxidants to prevent the oxidation of ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) to ABTS+ by Horseradish Peroxidase (HRP) and Hydrogen peroxide (H2O2). ABTS+, a soluble chromogen, can be monitored spectrophotometrically at 405 nm.

AT A GLANCE

Protocol summary

1. Prepare samples/Trolox (10 µL)
2. Add HRP working solution (20 µL)
3. Add ReadiUse™ ABTS Substrate Solution (150 µL)
4. Incubate at room temperature for 5 minutes
5. Monitor Absorbance at 405 nm

Important Equilibrate all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	405 nm
Recommended plate:	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Horseradish Peroxidase (HRP) stock solution (2000X):
Add 1 mL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component A).

2. Trolox standard solution (100 mM):
Add 100 µL of DMSO into the vial of Trolox (Component D).

PREPARATION OF STANDARD SOLUTION

Trolox standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15900

Prepare a Trolox standard by diluting 100 mM Trolox standard solution in assay buffer (Componenet B) using dilution factor =2 and the top concentration = 1 mM.

PREPARATION OF WORKING SOLUTION

HRP working solution (1X):
Add 2.5 uL of 2000X HRP stock solution into 5 mL of Assay Buffer (Component B) to make 1X HRP working solution.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Trolox standards and test samples in a clear 96-well microplate. SD = trolox standard (SD1 - SD7, 1 to 0.0156 mM), BL = blank control, TS = test sample.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	10 µL	serial dilution (1 to 0.0156 mM)
BL	10 µL	Assay Buffer (Component B)
TS	10 µL	sample

1. Prepare trolox standards (SD), blank controls (BL), and test samples (TS) (dilute the samples in Assay Buffer to bring the antioxidant level within the same range as Trolox, if necessary) according to the layout provided in Table 1 and Table 2.

2. Add 20 µL of HRP working solution into each well of Trolox standards, blank control, and test samples to make the total volume of 30 µL/well.

3. Add 150 µL of ReadiUse™ ABTS Substrate Solution (Component C) to each well, for a total volume of 180 µL/well.

   Note Avoid light and use in polypropylene containers.

4. Incubate for 5 minutes at room temperature.

   Note This 5 minutes incubation time is a guideline. The incubation time can be changed to obtain a more suitable absorbance.

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5. Read the absorbance at 405 nm using an absorbance microplate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance (405 nm)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Trolox samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

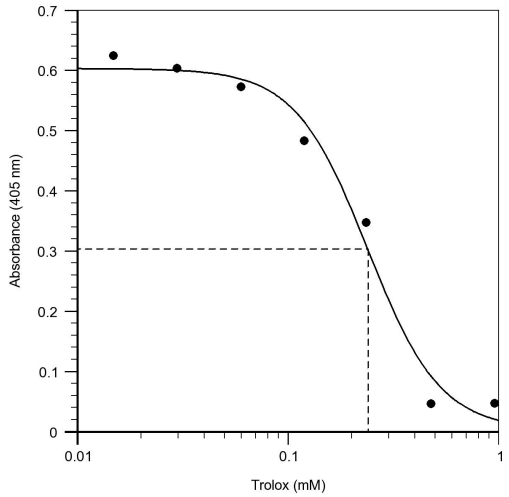

Figure 1. Trolox dose response was measured using Cell Meter Colorimetric Antioxidant Activity Assay Kit (Cat# 15900) in a 96-well clear plate using a SpectraMax microplate reader (Molecular Devices).

DISCLAIMER