ABSbio™ Antioxidant Detection Kit (K388-100)

Quantitative Colorimetric Ferric Reducing Antioxidant Power (FRAP) Assay

Introduction

Oxidative damage to living organisms has been associated with several disease states as well as aging. Antioxidants protect the cells from damages by reactive oxygen species which are produced in oxidation reactions in the cell. Antioxidants are also widely used as dietary supplements and in industry as preservatives in food. The ABSbio™ Antioxidant Detection Kit provides a simple, sensitive, one-step colorimetric assay to quantitatively measure antioxidant potential of a variety of samples including biological samples, food and drink (juice, coffee, wine & beer), agricultural and pharmaceutical products. The assay is based on the Ferric Reducing Antioxidant Power (FRAP) Assay in which iron reacts with a colorimetric probe to produce a colored complex by antioxidants. In this assay, antioxidant reduce Fe^{3+} to Fe^{2+} and react with co-factor to produce a blue colored product. The intensity of color, measured at 593 nm, is directly proportional to the total antioxidant power in the sample. The kit is supplied with sufficient reagents for 100 tests in 96-well plate assay, linear detection range of 5.7-1000 µM Fe^{2+}. It could easily be modified for use in 384-well assay and high-throughput assay.

Kit Components (100 tests)

Assay Buffer:	30 mL	Reagent A:	1 mL	Reagent B:	1 mL	Ferrous Standard (10 mM):	0.5 mL

Storage and Handling: Shipping on room temperature. Store kit at 4°C. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Serum, plasma, urine, or cell culture supernatant can be measured directly by a series of dilutions of the sample to ensure the readings are within the standard curve range. A variety of other samples including fruit juices, wine, extracts of tea, herbs, candies, drinks, and dried food extracts can be run in the kit after suitable dilution. Homogenize Cell (2 x 10^6) or tissue (20 mg) sample in 200 µL cold PBS. Centrifuge to collect the supernatant. It is recommended with all sample types to assay immediately or aliquot and store the samples at -20°C.
Transfer 20 µL sample into the 96-well clear flat bottom plate in duplicate. If sample is colored, 20 µL sample blank control needs to run parallel.

2. Standard Preparation

Transfer 25 µL 10 mM Ferrous standard into 225 µL assay buffer to generate a 1 mM Ferrous standard, then following the table to generate 1000, 600, 300, 100 and 0 µM standards.

Ferrous std(µL)	Assay buffer(µL)	final Ferrous concentration µM
50	0	1000
30	20	600
15	35	300
5	45	100
0	50	0

Transfer 20 µL of appropriate standards into the 96-well plate in duplicate. The blank control containing assay buffer only.

3. Reaction

Prepare enough working reagent by mixing 100 µL Assay buffer, 10 µL Reagent A, 10 µL Reagent B for each reaction (samples & standards). Transfer 100 µL prepared working reagent into each reaction well. **Note: Any significant blue color in the prepared working reagent may indicate contamination of the Assay Buffer, Reagent A or B.** Colored sample needs run sample blank control by directly add 90 µL assay buffer + 10 µL Reagent A to sample blank control well.

Tap plate to mix well. Incubate 30 min. at room temperature.

4. Measurement

Read the optical density at 593 nm (570-610 nm).

5. Calculation

Average the duplicate OD593 nm reading for standard and sample. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the Ferrous concentration of the standards. Determine the slope by linear regression and calculate the total antioxidant power of samples.

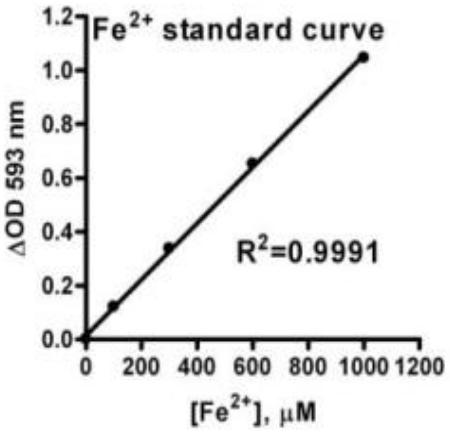
[Total antioxidant power**]= (**OD_{sample}-OD_{blank}**)/Slope x *n* (µM** Ferrous equivalent**)**

OD_{sample} and OD_{blank} are related optical density of the sample and assay buffer. *n* is the sample dilution factor.

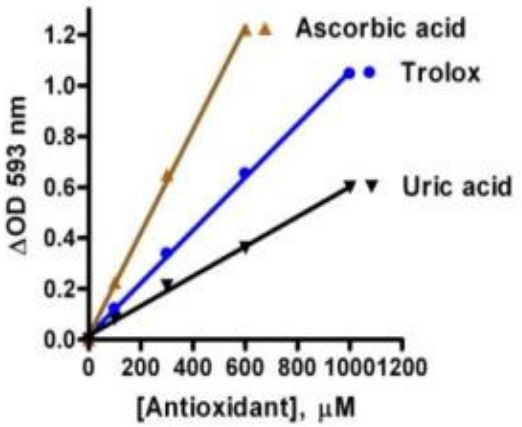
Note: For colored samples, correct for any sample interference by subtracting the sample blank reading from the sample reading.

If calculated Total antioxidant power (capacity) is higher than 1000 µM Ferrous equivalents, dilute sample in assay buffer and repeat assay. Multiply the results by the dilution factor.

Typical Standard Curve



Ferrous standard in 96 wells-plate assay (colorimetric). Always run your own standard curves for calculation of results.



Antioxidant power of Ascorbic acid & Uric acid

Sensitivity and Limit of Detection

The Limit of Detection was determined as 5.7 µM, and linear detection range up to 1000 µM Ferrous in 96-well plate colorimetric assay. Sensitivity was determined as 3.3 µM. Samples with values above 1000 µM Ferrous should be dilute with assay buffer, re-assayed, and multiply results by dilution factor.

Interferences

Culture media with phenol red, Triton x-100, Tween 20 were tested in the assay for interference. No significant change in the measured total antioxidant capacity level was observed. Solutions containing sodium azide will yield an instantaneous colored product with the reagents. No buffers or solutions containing azide can be measured using this kit.

References

Craft, BD, et al. 2012, Comprehensive Reviews in Food Science and Food Safety. 11:148-173
Benzie, IF. Strain, JJ. 1999, Method in Enzymology. 299:15–27.

Related Products:

Total Antioxidant Capacity Detection Kit (#K389-200)