Product Manual

OxiSelect™ Ferric Reducing Antioxidant Power (FRAP) Assay Kit

Catalog Number

STA-859 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Oxidative stress is a physiological condition where there is an imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. However, excessive ROS accumulation will lead to inflammation and cellular injury, such as damage to DNA, proteins, and lipid membranes. The cellular damage caused by ROS has been implicated in the development of many disease states, such as cancer, diabetes, cardiovascular disease, atherosclerosis, and neurodegenerative diseases. Under normal physiological conditions, cellular ROS generation is counterbalanced by the action of cellular antioxidant enzymes, macro or micro molecules, as well as other redox molecules. Antioxidants are molecules that contain one or more free electrons that can be donated to stabilize ROS. Antioxidants include both hydrophilic and lipophilic molecules for metabolizing ROS. These may be localized transiently within different tissues or cells. Because of their potential harmful effects, excessive ROS must be promptly neutralized and eliminated from the cells by this variety of antioxidant defense mechanisms.

Cell Biolabs' OxiSelect™ Ferric Reducing Antioxidant Power (FRAP) Assay Kit is a quantitative assay for measuring the antioxidant potential within various samples such as serum, plasma, urine, saliva, tears, tissue homogenates, cell extracts, and purified food or drug extracts. The assay is based on the highly-cited work of Benzie and Strain (ref. 1 and 2) in which iron reacts with a colorimetric probe to produce a blue product. The reaction is driven by the electron donating reducing power of antioxidants. The kit employs a ferrous iron standard which allows the user to determine the antioxidant content present within their sample. Each kit provides sufficient reagents to perform up to 200 assays, including standard curve and unknown samples. The FRAP assay offers a simple and efficient analytical method for assessing age, disease, diet, or other physiological changes to antioxidant status.

Assay Principle

The OxiSelect™ Ferric Reducing Antioxidant Power (FRAP) Assay Kit is a quantitative assay for measuring the antioxidant potential within a sample. Ferric iron (Fe^{3+}) is initially reduced by electron-donating antioxidants present within the sample to its ferrous form (Fe^{2+}). The iron-colorimetric probe complex develops a dark blue color product upon reduction which can be measured at 540-600 nm (see Figure 1). Samples can be compared to the iron standard for determining antioxidant potential. This assay is analytically sensitive to approximately 4 µM of Fe^{2+} iron equivalents, or a FRAP value of 2.

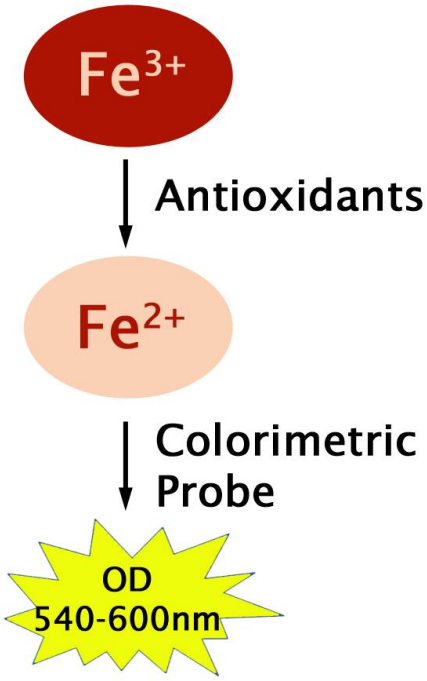

Figure 1. Assay Principle.

Related Products

1. STA-312: OxiSelect™ Total Glutathione Assay Kit
2. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
3. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
4. STA-341: OxiSelect™ Catalase Activity Assay Kit
5. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
6. STA-345: OxiSelect™ ORAC Activity Assay
7. STA-347: OxiSelect™ *In Vitro* ROS/RNS Assay Kit (Green Fluorescence)
8. STA-360: OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit
9. STA-802: OxiSelect™ *In Vitro* Nitric Oxide (Nitrite / Nitrate) Assay kit
10. STA-803: OxiSelect™ Myeloperoxidase Activity Assay Kit (Colorimetric)
11. STA-812: OxiSelect™ Glutathione Reductase Assay Kit
12. STA-860: OxiSelect™ Ascorbic Acid Assay Kit (FRASC) (Colorimetric)

Kit Components (shipped at room temperature)

1. Colorimetric Probe (Part No. 286001): Two 1 mL tubes
2. Iron Chloride Solution (Part No. 286002): Two 1 mL tubes
3. Iron (II) Standard (Part No. 285901): One 500 mg amber tube of crystals
4. Assay Buffer (5X) (Part No. 286004): Two 25 mL bottles

Materials Not Supplied

1. 96-well microtiter plates
2. 10 kDa MWCO centrifugal filter (for high protein content samples)
3. Ethanol or other organic solvent for lipid-based samples
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Conical tubes, microcentrifuge tubes, and bottles for sample and buffer preparation
7. Centrifuge and/or microfuge
8. Sonicator or tissue homogenizer
9. Multichannel micropipette reservoirs
10. Microplate reader capable of reading absorbance between 540-600 nm

Storage

Upon receipt, store the kit components at 4ºC.

Preparation of Reagents

- 1X Assay Buffer: Prepare 1X Assay Buffer by adding deionized water to the 5X Assay Buffer (e.g., add 3 mL of 5X Assay Buffer to 12 mL of deionized water). Mix thoroughly until homogeneous. Use this buffer for preparing kit reagents and within the assay. Store at 4ºC when not in use.
- Reaction Reagent: Prepare the Reaction Reagent just before use and prepare only enough for immediate applications. Dilute the Colorimetric Probe 1:10 and the Iron Chloride Solution 1:10 in 1X Assay Buffer (e.g., For 50 assays, add 500 µL Colorimetric Probe and 500 µL Iron Chloride Solution to 4 mL 1X Assay Buffer for 5 mL total). Vortex thoroughly.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. All samples should be assayed immediately or stored for up to 2 months at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. Samples may be diluted in 1X Assay Buffer as necessary before testing. Run proper controls as necessary. Always run a standard curve with samples.

- Serum*: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C.
- Plasma*: Collect blood sample and add to a blood collection tube containing heparin as the anticoagulant. Centrifuge at 3,000 rpm for 10-15 minutes at 4ºC. Remove the upper yellow

plasma supernatant layer without disturbing the white buffy coat (leukocytes). Samples should be tested immediately or frozen at -80°C.

**Note: Hemolyzed plasma or serum should be avoided. Heparinized plasma is recommended over EDTA plasma.*

- Cell lysates: Lyse 1-2 x 10^6 cells/mL by sonication or multiple freeze-thaw cycles in 4 volumes of cold 1X Assay Buffer. Centrifuge at 12,000 rpm for 15 minutes at 4ºC and remove insoluble cell material. A high concentration of protein may interfere with the assay. In this case, filter the sample with a 10kDa MWCO centrifugal filter before assaying (to reduce protein interference and turbidity). Test samples immediately or store at -80°C.
- Tissue lysates/homogenates: Homogenize/sonicate approximately 10 mg of tissue in 1-2 mL cold 1X Assay Buffer. Centrifuge the homogenate at 12,000 rpm for 15 minutes at 4ºC and collect the supernatant. A high concentration of protein may interfere with the assay. In this case, filter the sample with a 10kDa MWCO centrifugal filter before assaying (to reduce protein interference and turbidity). Test samples immediately or store at -80°C.
- Food extracts: Wash uncooked food (e.g., fruits or vegetables) in water and homogenize 5-10g (fresh wet weight) in 100 mL distilled water for 30 seconds. Filter homogenate and prepare dilutions in deionized water, 70% acetone, or 50% ethanol as necessary.

Preparation of Standard Curve

Note: The Iron (II) Standards should be prepared immediately prior to running the assay.

Prepare Iron (II) Standards fresh by weighing out the iron standard crystals for a 10 mg/mL solution in deionized water. This 10 mg/mL solution is equivalent to a concentration of 36 mM. Next, dilute this 36 mM stock to 1 mM in deionized water (e.g., add 125 µL of the 36 mM iron (II) solution to 4.375 mL deionized water). This is equivalent to a 1 mM or 1000 µM solution. Use this solution to prepare a series of standards according to Table 1 below. Prepare standards immediately prior to each assay performed. Vortex tubes thoroughly. Do not store or reuse the standard preparations.

Standard Tubes	Iron (II) 1000 µM (µL)	Deionized Water (µL)	Iron (II) Concentration (µM)
1	500	500	500
2	500 of tube #1	500	250
3	500 of tube #2	500	125
4	500 of tube #3	500	62.5
5	500 of tube #4	500	31.3
6	500 of tube #5	500	15.6
7	500 of tube #6	500	7.8
8	500 of tube #7	500	3.9
9	0	500	0

Table 1. Preparation of Iron (II) Standards.

Note: The Iron (II) Standard solutions are oxidized over time, yielding a yellow precipitate. Discard all iron solutions after use. Do not use iron solutions that contain precipitate.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each standard, sample and control should be assayed in duplicate or triplicate.
2. Add 100 µL of each standard, unknown sample or control to a 96-well plate.
3. Add 100 µL of the Reaction Reagent to all wells and mix by pipetting or with a horizontal shaker. Immediately time the reaction after adding the reagent and incubate 10 minutes at room temperature.

   Note: Reaction times may vary for some antioxidants. The assay reaction time can be extended, but a longer reaction time will result in higher background.
4. Immediately read absorbance of each well on a microplate reader using 540-600 nm as the primary wave length.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Calculate the net OD by subtracting the zero-standard value from samples and standards. This is the background correction.
3. Graph the standard curve (see Figure 2).
4. Compare the net OD of each sample to the standard curve to determine the quantity of antioxidant potential, as µM Fe^{2+} iron equivalents (FRAP value), present in the sample. Only use values within the range of the standard curve.

Example of Results

The following figures demonstrate typical FRAP Assay results at 540 nm. One should use the data below for reference only. This data should not be used to interpret actual results.

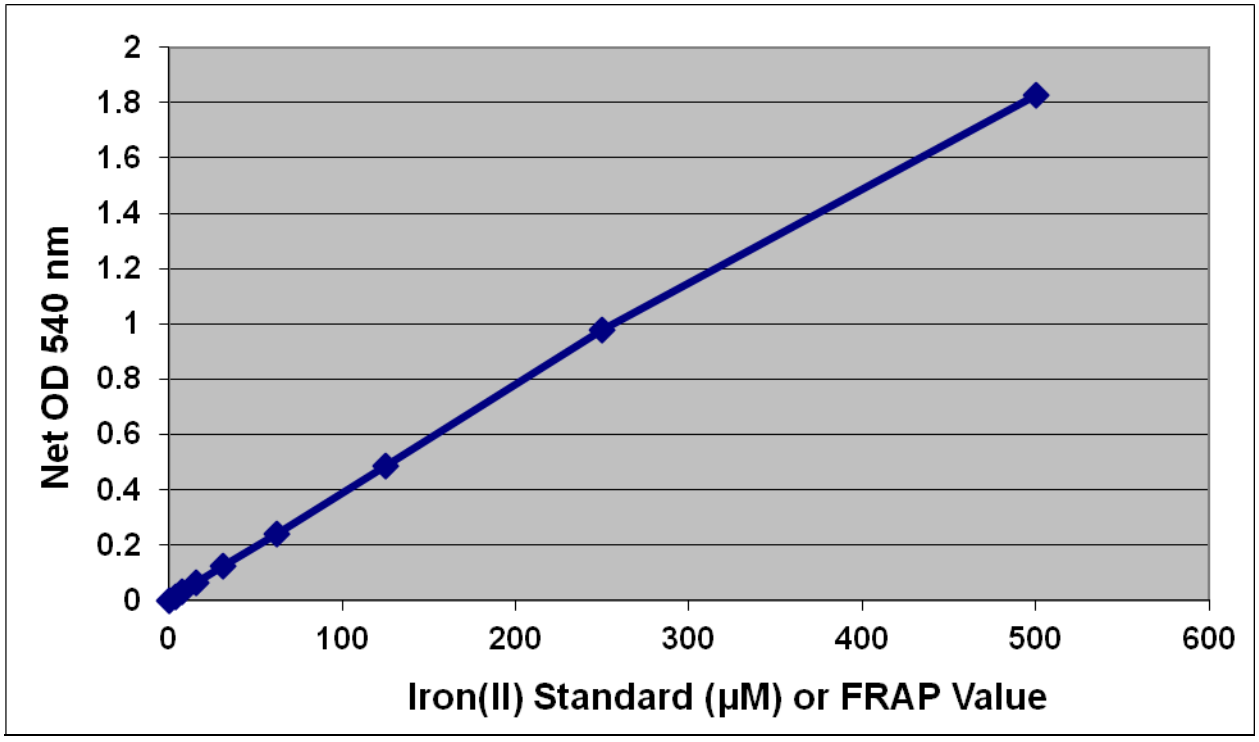

Figure 2. Iron (II) Standard Curve.

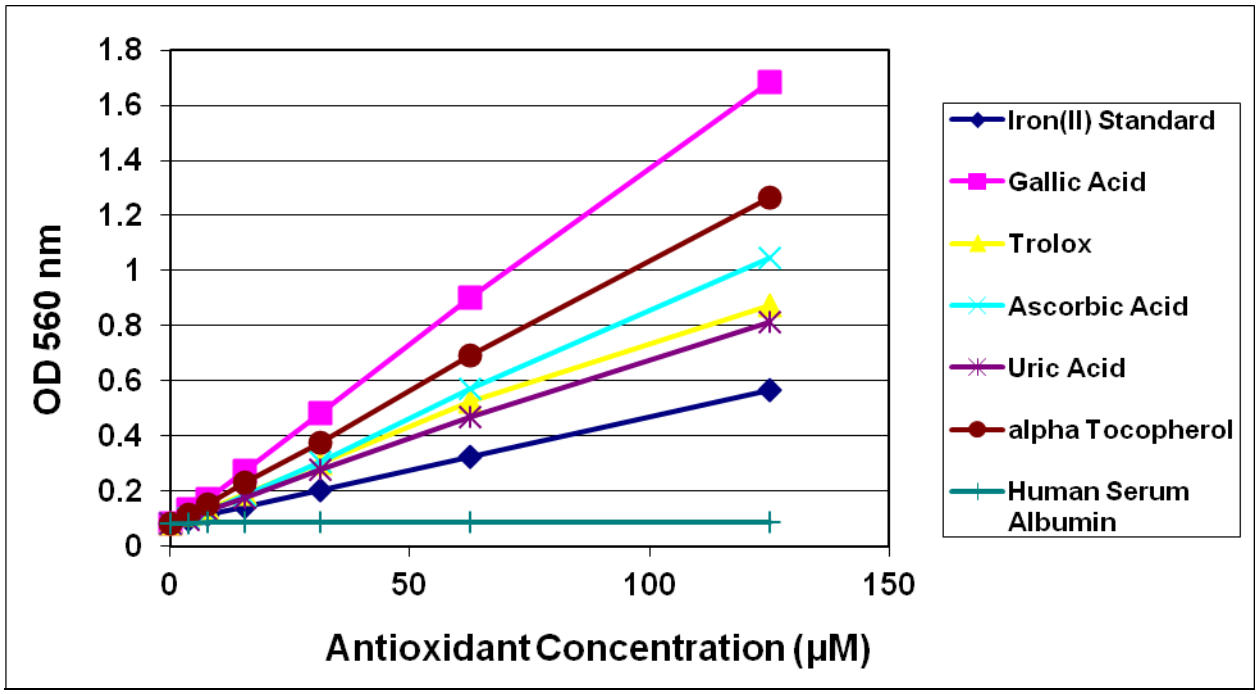

Figure 3. FRAP Assay results for various antioxidants.

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Recent Product Citations

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Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com