Product Manual

OxiSelect™ Ascorbic Acid Assay Kit (FRASC)

Catalog Number

STA-860 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Ascorbic acid is a vital water-soluble antioxidant found in living organisms. Commonly known as Vitamin C, the L-enantiomer is the only form found in nature and the form necessary for normal physiological reactions. Ascorbic acid is a weak sugar acid structurally related to glucose. The anion of ascorbic acid, ascorbate acts as a reducing agent, donating electrons to various enzymatic and some non-enzymatic reactions necessary in all animals and plants. Its reducing power makes it a powerful antioxidant, rapidly scavenging many reactive oxygen species (ROS). Although many organisms can produce the vitamin internally, humans cannot and must receive it through diet intake. Scurvy results when there is a deficiency in dietary intake of the vitamin. Ascorbic Acid is critical for a variety of functions related to tissue growth and wound healing, neurotransmitter formation, blood cholesterol levels, as well as free radical neutralization.

Cell Biolabs' OxiSelect™ Ascorbic Acid Assay Kit is a quantitative assay for measuring ascorbic acid within various samples such as serum, plasma, urine, saliva, tears, tissues homogenates, cell extracts, and purified food or drug extracts. The assay is based on the Ferric Reducing/Antioxidant Ascorbic Acid (FRASC) chemistry driven by the electron donating reducing power of antioxidants. The kit employs ascorbate oxidase, which allows the user to differentiate the ascorbic acid content from other antioxidants present within their sample. Ascorbic acid levels in a sample are determined by measuring the difference in optical density between two sample wells, one with and one without the enzyme. The FRASC assay is analytically sensitive to 1.56 µM; therefore, the kit offers a practical analytical method for assessing age, disease, diet, or other physiological changes to antioxidant status. Each kit provides sufficient reagents to perform up to 200 assays*, including standard curve and unknown samples.

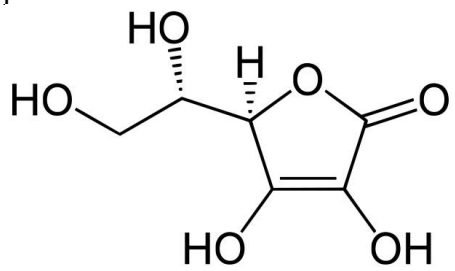

Figure 1. Ascorbic Acid Structure

Note: Each sample replicate requires 2 assays, one treated with ascorbic oxidase (+AO) and one without (-AO). Ascorbic acid is calculated from the difference in OD readings from the 2 wells.

Assay Principle

The OxiSelect™ Ascorbic Acid Assay Kit is a quantitative assay for measuring the ascorbic acid concentration within a sample. Ferric iron chloride is initially reduced by electron-donating antioxidants present within the sample to its ferrous form. The ferrous iron is chelated to a colorimetric probe to form a product that can be measured at 540-600 nm. Ascorbic acid reacts very quickly with the probe and iron complex, with the assay being complete within a few minutes. Ascorbate oxidase, added to one of two parallel sample wells, selectively and specifically destroys ascorbic acid. The background value generated is subtracted from wells untreated with the enzyme to determine the ascorbic acid concentration.

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)

Kit Components

Box 1 (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. Ascorbic Acid Standard (Part No. 286003): One 200 mg tube of powder
4. Assay Buffer (5X) (Part No. 286004): Two 25 mL bottles

Box 2 (shipped on blue ice packs)

1. Ascorbate Oxidase (50X) (Part No. 286005): One 40 µL tube

Materials Not Supplied

1. 96-well microtiter plates
2. 10 kDa MWCO centrifugal filter (for high protein content samples)
3. Ethanol
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, prepare single use aliquots and store the Ascorbate Oxidase at -80ºC. Store the remaining 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 to 4 mL 1X Assay Buffer for 5 mL total). Vortex thoroughly.
- 1X Ascorbate Oxidase: Prepare just before use and prepare only enough for immediate applications. Dilute 1:50 in deionized water and mix thoroughly (e.g., For 50 assays, add 10 µL of enzyme to 490 µL water).

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, as ascorbic acid is more stable in heparinized 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 5g (fresh wet weight) in 100 mL distilled water for 30 seconds. Filter homogenate and prepare dilutions in deionized water or 50% ethanol as necessary.

Preparation of Standard Curve

Note: The ascorbic acid standards should be prepared immediately prior to running the assay.

Prepare ascorbic acid standards fresh by weighing out 35 mg the Ascorbic Acid Standard powder and reconstituting it in 1 mL of deionized water. This is equivalent to a 200 mM ascorbic acid solution. Further dilute this solution 1:100 in 1X Assay Buffer for a 2 mM solution (e.g., add 5 µL of the 200 mM Ascorbic Acid Standard to 495 µL 1X Assay Buffer. Use this 2 mM 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	2 mM Ascorbic Acid (µL)	1X Assay Buffer (µL)	Final Ascorbic Acid Concentration (µM)
1	100	900	200
2	500 of tube #1	500	100
3	500 of tube #2	500	50
4	500 of tube #3	500	25
5	500 of tube #4	500	12.5
6	500 of tube #5	500	6.25
7	500 of tube #6	500	3.13
8	500 of tube #7	500	1.56
9	0	500	0

Table 1. Preparation of Ascorbic Acid standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each standard, sample and control should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with Ascorbic Oxidase (+AO) and one without the enzyme (-AO).
2. Add 100 µL of each ascorbic acid standard to 96-well plate wells.
3. Add 100 µL of each unknown sample or control to each of two separate wells.
4. Add 10 µL deionized water to all standard wells and one half of the paired sample wells (-AO).
5. Add 10 µL of the diluted 1X Ascorbate Oxidase to the remaining paired sample wells (+AO). Ensure that all wells are mixed thoroughly and incubate the plate 15 minutes at room temperature.
6. 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 2-3 minutes at room temperature.

   Note: Ascorbate reacts almost instantly under these experimental conditions while other antioxidants react much more slowly, so a longer reaction time will result in higher background.

7. 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. Subtract the average zero standard value from itself and all standard and sample values. This is the background correction.

3. Graph the standard curve (see Figure 2).

4. Subtract the sample well values containing Ascorbate Oxidase (+AO) from the untreated sample well values without Ascorbate Oxidase (-AO) to obtain the difference. The absorbance difference is due to ascorbic acid:

$$(\Delta A) = (A_{-AO}) - (A_{+AO})$$

5. Compare the change in absorbance (ΔA) of each sample to the standard curve to determine and extrapolate the quantity of ascorbic acid present in the sample. Only use values within the range of the standard curve.

Example of Results

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

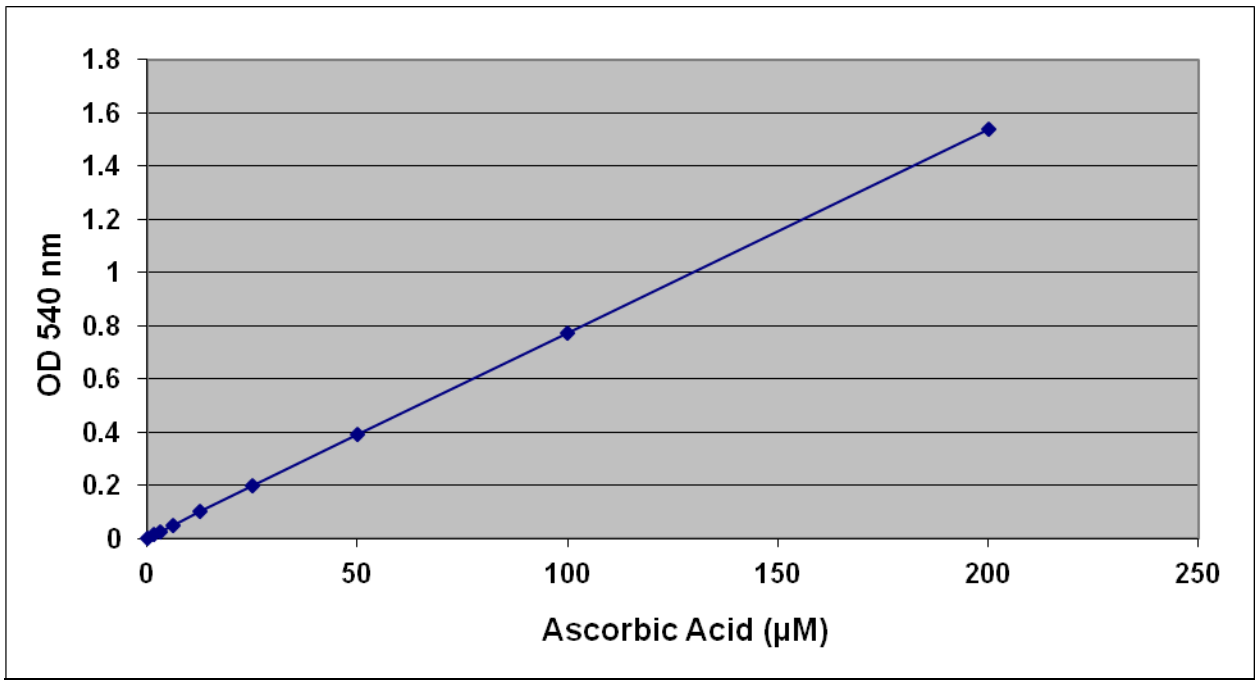

Figure 2. Ascorbic Acid Standard Curve.

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Recent Product Citations

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