EnzyChrom™ Ascorbic Acid Assay Kit (EASC-100)

Quantitative Colorimetric/Fluorometric Ascorbic Acid Determination

DESCRIPTION

Ascorbic acid (the *L*-enantiomer commonly known as vitamin C) is an important antioxidant found in living organisms and applied as additives in food and other industrial processes. By reacting with reactive oxygen species, it protects the cell from oxidative damages. BioAssay Systems' method provides a simple, direct and high-throughput assay for measuring ascorbic acid. In this assay, ascorbic acid is oxidized by ascorbate oxidase resulting in the production of H_2O_2 which reacts with a specific dye to form a pink colored product. The color intensity at 570nm or fluorescence intensity (530/585 nm) is directly proportional to the ascorbic acid concentration in the sample.

KEY FEATURES

Use 20 µL samples. Linear detection range: colorimetric assay 6 to 1000 µM, fluorimetric assay 1 to 100 µM ascorbic acid.

APPLICATIONS

Assays: ascorbic acid in biological samples such as serum, plasma, urine, saliva, milk, tissue, and cell culture.

Drug Discovery/Pharmacology: effects of drugs on ascorbic acid metabolism.

KIT CONTENTS

Assay Buffer: 10 mL **Enzyme Mix:** 120 µL
Dye Reagent: 120 µL **Standard**: 400 µL 10 mM ascorbic acid

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIMETRIC ASSAY

Note: SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol, > 5 µM) are known to interfere in this assay and should be avoided in sample preparation.

Sample treatment: liquid samples such as serum and plasma can be assayed directly. Tissue and cell (10^6-10^7) lysates can be prepared by homogenization in cold 1 x PBS and centrifugation (5 min at 14,000 rpm). Use clear supernatants for assay. Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor n = 1.36).

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.
2. *Standards*: mix 22 µL 10 mM Standard with 198 µL dH_2O (final 1000 µM). Dilute standard in dH_2O as follows.

No	1000 µM STD + H_2O	Vol (µL)	Ascorbic acid (µM)
1	100 µL + 0 µL	100	1000
2	60 µL + 40 µL	100	600
3	30 µL + 70 µL	100	300
4	0 µL +100 µL	100	0

Transfer 20 µL diluted standards into separate wells of a clear flat-bottom 96-well plate.

Samples: transfer 20 µL of each sample into separate wells of the plate.

3. Color r*eaction*. Prepare enough Working Reagent by mixing, for each reaction well, 85 µL Assay Buffer, 1 µL Enzyme Mix and 1 µL Dye Reagent. Add 80 µL Working Reagent to each well. Tap plate to mix. Incubate 10 min at room temperature.

4. Read optical density at 570nm (550-585nm).

FLUORIMETRIC ASSAY

The fluorimetric assay procedure is similar to the Colorimetric Assay except that (1) 0, 30, 60 and 100 µM ascorbic acid standards and (2) a black 96-well plate are used. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 585 nm.

Note: if the calculated Ascorbic acid concentration of a sample is higher than 1000 µM in the Colorimetric Assay or 100 µM in the Fluorimetric Assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor n.

CALCULATION

Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the ascorbic acid concentration of Sample,

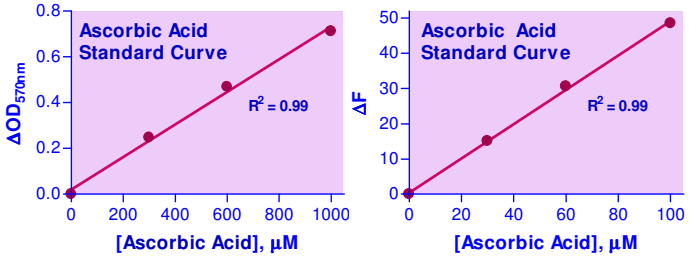
$$[\text{Ascorbic Acid}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \qquad (\mu M)$$

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and H_2O Blank, respectively. n is the sample dilution factor.
Conversions: 1 mM ascorbic acid equals 17.6 mg/dL, 0.0176% or 176 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader; black flat-bottom uncoated 96-well plates, fluorescence plate reader.

Ascorbic acid Standard Curves



96-well colorimetric assay ***96-well fluorimetric assay***

LITERATURE

1. Baker, W.L. and Lowe, T. (1985). Sensitive ascorbic acid assay for the analysis of pharmaceutical products and fruit juices. Analyst 110:1189-1191.

2. Chung, W.Y. et al (2001). Plasma ascorbic acid: measurement, stability and clinical utility revisited. Clin Biochem. 34:623-627.

3. Arya, S.P. et al (2002). A new method for the ascorbic acid assay using iron(II)-pyridine-2,6-dicarboxylic acid complex. Ann Chim. 92: 1159-1164.

Ascorbic Acid Assay Using Handheld Fluorometer (Cat# FL530590)

DESCRIPTION

Ascorbic acid (the *L*-enantiomer commonly known as vitamin C) is an important antioxidant found in living organisms and applied as additives in food and other industrial processes. By reacting with reactive oxygen species, it protects the cell from oxidative damages. BioAssay Systems' method provides a simple and direct assay for measuring ascorbic acid using the handheld fluorometer. In this assay, ascorbic acid is oxidized by ascorbate oxidase resulting in the production of H_2O_2 which reacts with a specific dye to form a pink colored product. The color intensity at 570nm or fluorescence intensity (530/585 nm) is directly proportional to the ascorbic acid concentration in the sample.

KEY FEATURES

Assay Performance.

Linear Detection Range: 1 to 100 µM

Simple and convenient. The procedure involves addition of a single working reagent and incubation for 10 min at room temperature.

Samples: serum, plasma, and other biological samples.

APPLICATIONS

Assays: ascorbic acid in biological samples such as serum, plasma, urine, saliva, milk, tissue, and cell culture.

KIT CONTENTS

Assay Buffer: 10 mL **Enzyme Mix:** 120 µL
Dye Reagent: 120 µL **Standard**: 400 µL 10 mM ascorbic acid

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

Important: prior to assay, bring the assay reagents to room temperature. Keep the Enzyme Mix on ice or in refrigerator during the assays.

1. Prepare 100 µM ascorbic acid standard by mixing 5 µL provided Standard with 495 µL H2O.

In separate mini-glass tubes, add 20 µL H_2O ("Blank"), 20 µL 100 µM Standard ("Std"), and 20 µL Sample.

2. Prepare enough Working Reagent by mixing, for each tube: 85 µL Assay Buffer, 1 µL Enzyme Mix and 1 µL Dye Reagent.

Add 80 µL Working Reagent to each tube. Mix by vortexing or pipetting.

Incubate 10 min at room temperature in the dark.

3. Switch on the reader. To calibrate the reader, place the "Blank" tube into the sample holder. Press "Calibrate", "Assay 1", then "Blank". Reader starts Measuring.

Press the arrow on "<- Std -> ", until the window shows "100".

Place the “Std” tube into the Sample holder. Press "Measure".

The reader shows "Calibrate Finished". The Reader is now calibrated.

Press "Return".

4. Measure. Place the sample tube into the Sample Holder.

Press "Measure" → "Assay 1" → "Measure".

The ascorbic acid concentration will be displayed in the window. Record the data, or press "Save" to save the data for later retrieval.

Press "Return" and then "Measure" for the next sample.

Note: if Sample concentration is higher than the upper limit, dilute Sample in H_2O and repeat assay.

Conversions: 1 mM ascorbic acid equals 17.6 mg/dL, 0.0176% or 176 ppm.