CREATIVE BIOMART®
Assay Kit

Ascorbic Acid Assay Kit

Product Information

Cat.No. Kit-0105

Product Overview

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. Kit-0105 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 H2O2 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.

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.

Storage

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

Size

100 tests

Kit Components

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

Materials Required but Not Supplied

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.

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Detection method OD570nm, or FL530/585nm

Compatible Sample Types

Serum, plasma, urine, saliva, milk, tissue, and cell culture

Features & Benefits

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

Assay Protocol

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 dH2O (final 1000 µM). Dilute standard in dH2O as follows.
No 1000 µM STD + H2O 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 flatbottom 96-well plate.
Samples: transfer 20 µL of each sample into separate wells of the plate.
3. Color reaction. 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

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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 λex = 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.

Analysis

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,
[Ascorbic Acid] = (R SAMPLE – R BLANK)/ Slope (µM-1) × n (µM)
R SAMPLE and R BLANK are optical density or fluorescence intensity readings of the Sample and H2O Blank, respectively. n is the sample dilution factor.
Conversions: 1 mM ascorbic acid equals 17.6 mg/dL, 0.0176% or 176 ppm.