Vitamin C (VC) Content Assay Kit (Phenanthroline, Microassay Method)

V1515893

Storage 2-8℃. Protect from light.

Shipping Transported under low temperature. Store immediately under the required storage conditions upon receipt.

Product Introduction

Vitamin C, also known as L-ascorbic acid, is an essential nutrient for higher primates and a few other organisms. In living organisms, vitamin C acts as an antioxidant, an acidic hexose derivative, and an enediol hexonic acid lactone, protecting the body from free radical damage. It also functions as a coenzyme and is widely found in various fresh fruits and vegetables. Vitamin C exists in two isomeric forms: L-form and D-form. Only the L-form has physiological functions, and both the reduced form and oxidized form exhibit biological activity.

The detection principle of the Vitamin C Assay Kit (Phenanthroline, Microassay Method) is as follows: Under acidic conditions, vitamin C reduces ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which then form a stable red chelate complex with phenanthroline. The absorbance is measured at 534 nm using a microplate reader. Within a certain concentration range, the absorbance shows a linear relationship with the vitamin C content, allowing the determination of vitamin C concentration.

Components

V1515893	Component	48 T	96 T	Storage conditions
V1515893A	Extraction Buffer(5×)	10 mL	20 mL	2-8℃. Store in the dark
V1515893B	Acidic Buffer	1.5 mL	3 mL	2-8℃
V1515893C	Assay Reagent A	15 mg	25 mg	2-8℃. Store in the dark
V1515893D	Assay Reagent B	1 mL	2 mL	2-8℃. Store in the dark
V1515893E	Vc Standard	2×5 mg	4×5 mg	2-8℃. Store in the dark

Usage Protocol

1. Reagent Preparation

1) Equilibrate all reagents in the kit to room temperature.
2) Prepare 1× Extraction Solution by mixing Extraction Solution (5×) and double-distilled water in a volume ratio of 1:4. Store at 2-8°C protected from light.
3) Before Add 5 mL (for 96 tests) or 3 mL (for 48 tests) of absolute ethanol to Detection Reagent A and mix well. Store at 2-8°C protected from light.
4) Take one vial of Vitamin C standard, add 1 mL of 1× Extraction Solution to obtain a 5 mg/mL Vitamin C standard solution. Then dilute it with 1× Extraction Solution to a 250 μg/mL Vitamin C standard solution. Dilute according to the table below. Prepare fresh before use:

Volume of 250 μg/mL Vitamin C Standard (μL)	1.6	8	16	24	32	40
Volume of 1× Extraction Solution (μL)	38.4	32	24	16	8	0
Final Vitamin C Concentration (μg/mL)	10	50	100	150	200	250

2. Sample Preparation

1) Samples must not contain reducing agents such as DTT or 2-mercaptoethanol, nor chelating agents such as HEDP or EDTA.
2) Accurately weigh the tissue sample, add 9 volumes of 1× Extraction Solution according to the weight (g): volume (mL) ratio of 1:9, grind and homogenize. Collect the supernatant after centrifugation at 4000 g for 10 minutes. The supernatant is the test solution.

3. Assay Procedure

1) Perform a preliminary assay on 2-3 samples with expected large differences before formal measurement.
2) Preheat the microplate reader for 30 minutes and set the wavelength to 534 nm.
3) Add reagents sequentially according to the table below:

Reagent (μL)	Blank Well	Standard Well	Sample Well
1× Extraction Buffer	40	-	-
Vitamin C Standard	-	40	-
Test Sample	-	-	40
Deionized Water	80	80	80
Acidic Buffer	20	20	20
Reagent A	40	40	40
Reagent B	20	20	20

Mix well and incubate at 30°C for 30 minutes. Measure the absorbance at 534 nm in a 96-well

microplate.

4. Result Calculation

Plot a standard curve with the series of standard vitamin C concentrations (0, 10, 50, 100, 150, 200, 250 μg/mL) as the x-axis and the corresponding absorbance values as the y-axis to obtain the regression equation. Substitute the absorbance of the sample well into the regression equation to calculate the vitamin C concentration.

Vitamin C Content (μg/g) = Csample × Vsample × N / W

C_{sample}: Vitamin C concentration obtained from the standard curve (μg/mL);

V_{sample}: Total volume of sample extraction solution (μL);

N: Sample dilution factor;

W: Sample mass (g).

5. Precautions

1) If the sample concentration is too high, dilute it with distilled water and re-measure. Multiply the result by the dilution factor.
2) Vitamin C standard is easily oxidized. Therefore, it is recommended to prepare the standard solution after the sample supernatant is prepared, and perform the assay within 10 minutes.

Result Presentation

Typical Standard Curve: