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Vitamin C (VC) Assay Kit

Catalog No.: BC00036

Size: 50T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Vitamin C (VC) Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma, animal and plant tissues
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (530-540 nm, optimal detection wavelength 536 nm)
Range	0.625-20μg/mL
Sensitivity	0.31μg/mL

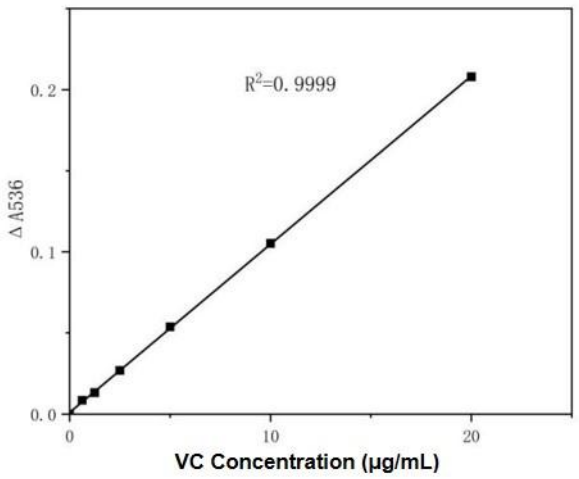
Product Introduction

Vitamin C, as a key antioxidant, plays multiple physiological roles in the body, including promoting collagen synthesis, enhancing iron absorption, and neutralizing free radicals. Accurate determination of vitamin C levels is essential for assessing an individual's nutritional status and overall antioxidant capacity.

Detection Principle

In the presence of phenanthroline, VC can reduce trivalent iron ions to divalent iron ions, which can form a pink complex with phenanthroline under certain conditions, with a maximum absorption wavelength at 536 nm. The content of VC can be calculated by colorimetry.

When this kit is used to test tissue samples, the total protein concentration needs to be measured. We recommend the protein concentration determination kit (BCA method) (BC00006) produced by EnkiLife.



Product composition

Serial Number	Product Name	Packing specification (50T)	Storage
Reagent 1	Extract	20 mL	-20℃, avoid light, store at 2-8℃
Reagent 2	Buffer	8 mL	-20℃, store at 2-8℃ after opening
Reagent 3	Color developer	15mL	-20℃, avoid light, store at 2-8℃
Reagent 4	Iron reagent	0.3 mL	-20℃, avoid light, store at 2-8℃
Reagent 5	VC Standard	6 mg	-20℃, avoid light, store at 2-8℃
Consumables 1	96-well ELISA	1 plate	RT
Consumables 2	96-well	2 pieces	RT

Storage conditions

The unopened kit can be stored at -20℃ for 12 months. After opening, it can be stored at 4℃ for 6 months.

Preparation before the experiment

Sample processing

1. Sample requirements: The sample cannot contain reducing reagents such as DTT and 2-mercaptoethanol, and cannot contain chelating agents such as HEDP and EDTA.
2. Serum (plasma) samples: can be measured directly. Tissue samples: routine homogenization, use physiological saline (0.9% NaCl solution) or PBS (0.01 M, pH 7.4) as the homogenate. After homogenization, centrifuge at 4°C, 10,000×g for 10 min, take the supernatant and place it on ice for testing. Keep part of the supernatant for protein concentration determination.

· Preparation of the kit

1. Equilibrate the kit to room temperature before testing.
2. Preparation of reagent 3 working solution: dilute reagent 3 with ethanol in a ratio of 1:9 and store at 2-8 ℃ away from light for 7 days.
3. Preparation of reagent 4 working solution: Take 0.05mL of reagent 4 and dilute it to 1 with double distilled water. mL is enough and it can be stored at 2-8℃ away from light for 7 days.
4. Preparation of 60 μg/mL standard: Take one vial of reagent 5, add 1 mL of reagent 1 to dissolve it, the concentration is 6 mg/mL , then take an appropriate amount and dilute it 100 times with reagent 1, which is the 60 μg/mL standard. Since the standard is easily oxidized, it is necessary to prepare the standard after the sample supernatant is prepared, and the experiment should

be carried out within 10 minutes .

5. Dilution of different concentrations of standard : dilute the 60 μg/mL standard solution into different concentrations using reagent 1 by half dilution method. 20, 10, 5, 2.5 , 1.25, 0.625 , 0 (blank well) μg /ml .

Operation process

1. Preparation of supernatant: Take 0.10 mL of the sample to be tested into a 2 mL EP tube, add 0.30 mL of reagent 1 working solution, vortex mix, let stand at room temperature for 15 minutes, centrifuge at 2000×g for 10 minutes, and take the supernatant for testing. That is, it is diluted 4 times here.
2. Standard tube: 2 Add 100 mL EP tube μL standard; assay tube: at 2 Add 100 mL EP tube μL of the sample supernatant prepared in step (1) .
3. 2 to the standard tube and sample tube in step 2, 250 μL reagent three working solution , 65 μL of reagent IV working solution, vortex thoroughly to mix.
4. Incubate at 37°C for 30 min, let stand at room temperature for 5 min, then take 250 Add μL to the ELISA plate, measure the OD value of each well at 530-540 nm (optimal 536 nm).

	Standard tube	**Determination tube**	**Blank tube**
Standard solutions of different	100	--	--
Reagent 1 (μL)	--	--	100
Supernatant of sample to be	--	100	--
Reagent 2 (μL)	125	125	125
Reagent 3 (μL)	250	250	250
Reagent 4 working solution (μL)	65	65	65
Vortex to mix, and incubate at 37°C for 30 min, let stand at room temperature for 5 min and then take 250 μL was added to the ELISA plate, 530 -540 nm (optimal 536 nm) , and measure the OD value of each well.			

Result calculation

Standard fitting curve: y = ax + b

Calculation formula for VC content in serum (plasma) :

VC content (μg/mL) = $(\Delta A_{536} - b) \div a \times f \times 4^{*}$ (μ g/mL)

Calculation formula for VC content in tissue samples :

VC content (μg/mL) = $(\Delta A_{536} - b) \div a \times f \div C_{pr} \times 4^{*}$ (μg / m gprot)

annotation :

y: OD value of standard sample minus OD value of blank sample

x: concentration of the standard

a: slope of the curve

b: intercept of the curve

ΔA_{536} : Sample OD value - Blank OD value

f: dilution factor of the sample before adding it to the detection system

C_{pr} : protein concentration of the sample to be tested (mgprot /L)

4^{*} : Dilution factor of the sample during supernatant preparation (4 times)

Notes

1. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
2. Since the standard products in this kit are easily oxidized after preparation, please prepare the samples first and then the standard products. The prepared standard products should not be left for too long.
3. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
4. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.