（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K034-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometer (536 nm)

Detection range：0.35-20 μg/mL

Elabscience® Vitamin C (VC) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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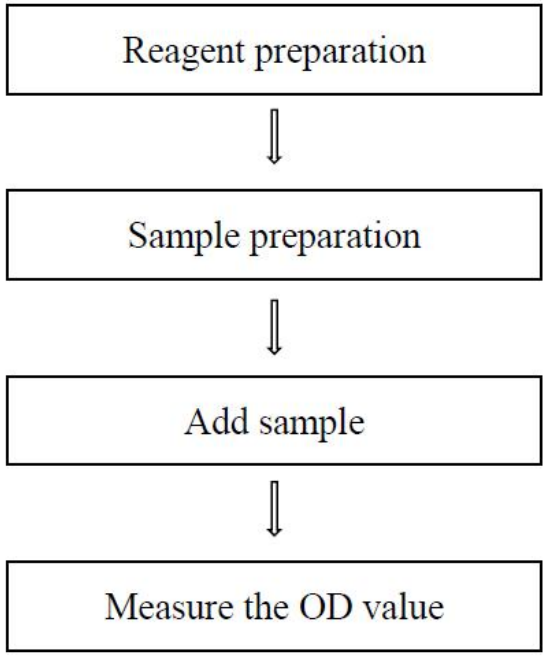
Assay summary



Intended use

This kit can be used to measure Vitamin C content in serum, plasma, animal/plant tissue samples.

Detection principle

The most obvious chemical activity of Vitamin C (VC) is that reduce Fe^{3+} to Fe^{2+}, then promote iron absorption in the intestine, promote the storage and utilization of iron. Fe^{3+} react immediately with reducing ascorbic acid to form Fe^{2+}. Then Fe^{2+} react with phenanthroline and the color developing reaction occurs. The content of VC in sample can be determined. Measure the OD value and calculate the VC content indirectly.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Extracting Solution	10 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 months shading light
Reagent 2	Buffer Solution	25 mL × 1 vial	50 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	6 mL ×1 vial	12 mL ×1 vial	2-8℃, 12 months shading light
Reagent 4	Ferrum Reagent	1 mL × 1 vial	1 mL × 1 vial	2-8℃, 12 months shading light
Reagent 5	Stop Solution	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months
Reagent 6	VC Standard	6 mg × 3 vials	6 mg × 3 vials	2-8℃, 12 months shading light

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (536 nm), Micropipettor, Vortex mixer, Incubator, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), Absolute ethanol

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of extracting application solution:

Before testing, please prepare sufficient extracting application solution according to the test wells. For example, prepare 1.2 mL of extracting application solution (mix well 80 μL of the extracting solution with 1120 μL of double distilled water). Store at 2-8°C for 7 days protected from light.

③ The preparation of chromogenic application solution:

For each tube, prepare 1 mL of chromogenic application solution (mix well 0.1 mL of chromogenic agent and 0.9 mL of absolute ethanol). Store at 2-8°C for 7 days protected from light.

④ The preparation of ferrum application solution:

Dilute 0.15 mL of the ferrum reagent with double distilled water to a final volume of 25 mL. The ferrum application solution should be prepared on spot.

⑤ The preparation of 6 μg/mL standard solution:

Dissolve one vial of VC standard with 6 mL of extracting application solution to prepare 6 mg/mL standard solution.

Before testing, please prepare sufficient 6 μg/mL standard solution according to the test wells. For example, prepare 0.4 mL of 6 μg/mL standard solution (mix well 0.4 μL of 6 mg/mL standard solution and 399.6 μL of extracting application solution).

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human plasma	1
10% Mouse kidney tissue homogenization	1
Rat serum	1
Mouse serum	1
10% Rat liver tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The sample pretreated with extracting solution must be clarified after centrifugation.

② Standard is easy to oxidized, it is best to use within 10 min.

Operating steps

The pretreatment of sample supernatant:

Take 0.15 mL of sample, add 0.45 mL of extracting application solution, mix fully with a vortex mixer and stand for 15 min at room temperature, then centrifuge at 2000 g for 10 min. Take the supernatant for detection.

The measurement of samples:

① Blank well: add 0.4 mL of extracting application solution to the 5 mL EP tube.
Standard well: add 0.4 mL of 6 μg/mL standard solution to the 5 mL EP tube.
Sample well: add 0.4 mL of the supernatant in sample preparation step to the 5 mL EP tube.

② Add 0.5 mL of buffer solution, 1 mL of chromogenic application solution and 0.25 mL of ferrum application solution to each tube.

③ Mix well with a vortex mixer and incubate at 37℃ for 30 min.

④ Add 0.1 mL of stop solution and mix well with a vortex mixer.

⑤ Stand for 10 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 536 nm with 1 cm optical path cuvette.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{VC content (μg/mL)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \times 4^*$$

2. Tissue sample:

$$\text{VC content (μg/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr} \times 4^*$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 6 μg/mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL.

4*: Dilution factor of sample preparation, 4 times.

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Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	1.20	9.50	15.60
%CV	2.5	2.2	2.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	1.20	9.50	15.60
%CV	3.6	2.9	3.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μg/mL)	2.5	8.5	13.5
Observed Conc. (μg/mL)	2.5	9.0	14.3
Recovery rate (%)	100	106	106

Sensitivity

The analytical sensitivity of the assay is 0.07 μg/mL VC. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

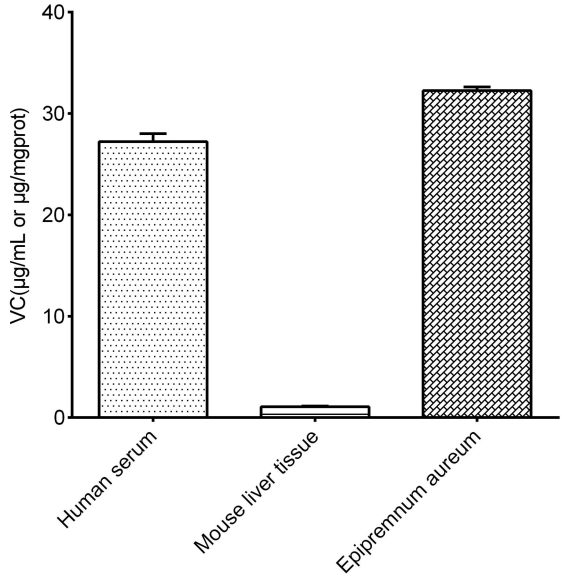
Example analysis:

Take 0.3 mL of human serum, add 0.9 mL of reagent 1 application solution, mix fully with a vortex mixer and stand for 15 min, then centrifuge at 2000 g for 10 min, take the supernatant for detection and carry the assay according to the operation steps. The results are as follows:

the average OD value of the sample is 0.284, the average OD value of the blank is 0.049, the average OD value of the stdandard is 0.256, the concentration of standard is 6 μg/mL, and the calculation result is:

$$\text{VC content (μg/mL)} = \frac{0.284 - 0.049}{0.256 - 0.049} \times 6 \times 4 = 27.2 \text{ μg/mL}$$

Detect human serum, 10% rat liver tissue homogenate (the concentration of protein in sample is 8.52 mgprot/mL), 10% Epipremnum aureum tissue homogenate (the concentration of protein in sample in is 2.45 mgprot/mL) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.