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

Catalog No：E-BC-K034-M

Specification：48T(34 samples)/96T(82 samples)

Measuring instrument：Microplate reader(530-540 nm)

Detection range：0.31-17.5 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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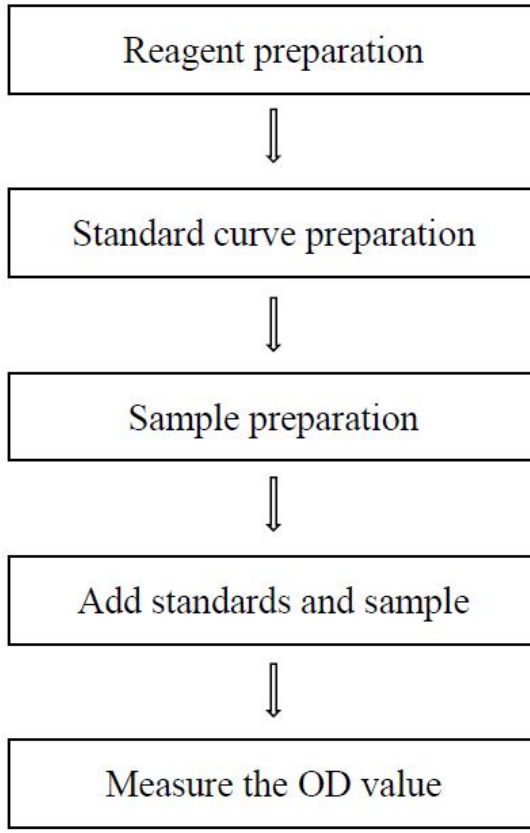
Assay summary



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(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	2 mL × 1 vial	5 mL × 1 vial	2-8℃, 12 months shading light
Reagent 2	Buffer Solution	8 mL × 1 vial	15 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	Ferrum Reagent	0.5 mL × 1 vial	0.5 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 × 2 vials	6 mg × 2 vials	2-8℃, 12 months shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

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:

Microplate reader (530-540 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

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 working solution:

Before testing, please prepare sufficient extracting application solution according to the test wells. For example, prepare 4.5 mL of extracting application solution (mix well 0.3 mL of the extracting solution with 4.2 mL of double distilled water). Store at 2-8°C for 7 days protected from light.

③ The preparation of chromogenic application solution:

For each well, prepare 250 μL of chromogenic application solution (mix well 25 μL of chromogenic agent and 225 μL 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. Store at 2-8°C for 7 days protected from light.

⑤ The preparation of 6 mg/mL VC standard solution:

Dissolve one vial of VC standard with 1 mL of extracting working solution to prepare 6 mg/mL VC standard solution.

⑥ The preparation of 60 μg/mL VC standard solution:

Before testing, please prepare sufficient 60 μg/mL VC standard solution according to the test wells. For example, prepare 700 μL of 60 μg/mL VC

standard solution (mix well 7 μL of 6 mg/mL VC standard solution and 693 μL of extracting working solution).

Note: VC standard is easy to oxidized, it is best to use 60 μg/mL VC standard solution within 10 min.

⑦ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 60 μg/mL VC standard solution with extracting working solution diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 7.5, 10, 12.5, 15, 17.5 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦
Concentration (μg/mL)	**0**	**5.0**	**7.5**	**10.0**	**12.5**	**15.0**	**17.5**
60 μg/mL VC standard (μL)	0	50	75	100	125	150	175
Extracting working solution (μL)	600	550	525	500	475	450	425

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
Mouse serum	1
Chicken serum	1
Horse serum	1
10% Mouse kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Plant tissue homogenate	1

Human serum	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 VC standard is easy to oxidized, please prepared freshly.

② In the step of pretreatment of sample supernatant, the supernatant after centrifugation should be clarified.

Operating steps

The pretreatment of sample supernatant:

Take 0.10 mL of sample, add 0.30 mL of extracting working solution, mix well with vortex mixer and stand for 15 min at room temperature, then centrifuge at 2000×g for 10 min. Take the supernatant for test.

The measurement of samples:

① Standard well: add 100 μL of standard solution with different concentrations to the 2 mL EP tubes.

Sample well: add 100 μL of sample supernatant to the 2 mL EP tubes.

② Add 125 μL of buffer solution, 250 μL of chromogenic application solution and 65 μL of ferrum application solution.

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

④ Add 25 μL of stop solution and mix well with a vortex mixer.

⑤ Stand for 10 min at room temperature. Take 250 μL of reaction solution to microplate and measure the OD value at 536 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

$$\text{VC content (μg/mgprot)} = (\Delta A_{536} - b) \div a \times f \times 4^{*} \div Cpr$$

[Note]

f: Dilution factor of sample before tested.

ΔA_{536}: $OD_{Sample} - OD_{Blank}$.

4*: Dilution factor of the pretreatment of sample supernatant.

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

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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)	1.50	8.40	12.50
%CV	2.7	2.1	1.8

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)	1.50	8.40	12.50
%CV	5.8	6.4	6.1

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 108%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	6.5	11	13.5
Observed Conc. (μg/mL)	7.2	11.6	14.7
Recovery rate (%)	110	105	109

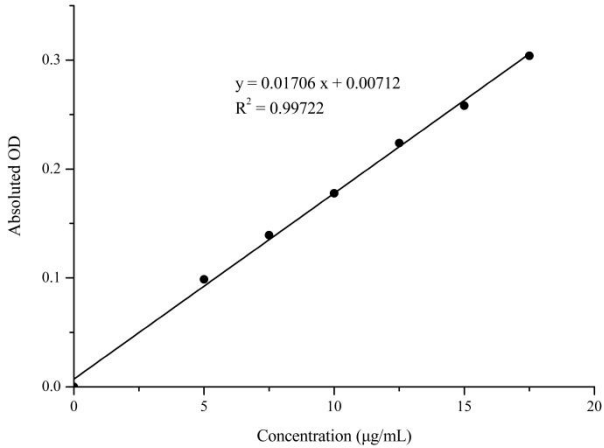
Sensitivity

The analytical sensitivity of the assay is 0.31 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μg/mL)	0	5	7.5	10	12.5	15	17.5
Average OD	0.054	0.152	0.193	0.231	0.278	0.312	0.358
Absoluted OD	0	0.098	0.139	0.177	0.224	0.258	0.304



Appendix II Example Analysis

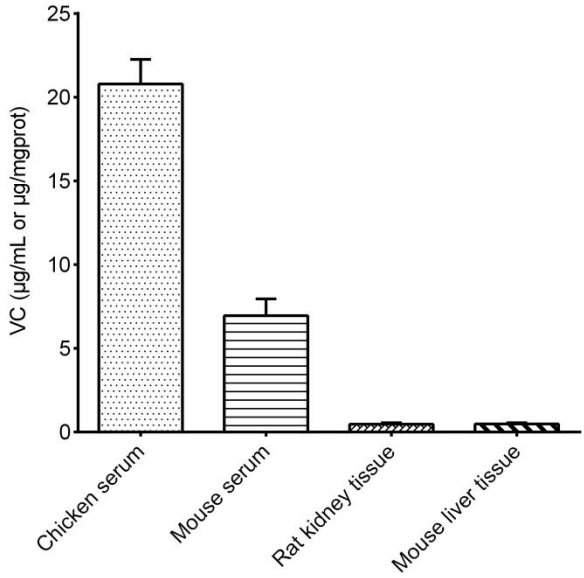
Example analysis：

Take 0.1 mL of chicken serum, add 0.30 mL of extracting application solution, mix fully with vortex mixer and stand for 15 min at room temperature, centrifuge at 2000 g for 10 min, then take the supernatant and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0233 x + 0.0198, the average OD value of the sample is 0.203, the average OD value of the blank is 0.062, and the calculation result is:

$$\text{VC content (μg/mL)} = (0.203 - 0.062 - 0.0198) \div 0.0233 \times 4 = 20.81 \text{ μg/mL}$$

Detect chicken serum, mouse serum, 10% rat kidney tissue homogenate (the concentration of protein in sample is 10.17 mgprot/mL), and 10% mouse liver tissue homogenate (the concentration of protein in sample is 13.01 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.