PRODUCT INFORMATION & MANUAL

Vitamin C/Ascorbic Acid Assay Kit (Colorimetric)

NBP3-25906

For research use only.
Not for diagnostic or therapeutic procedures.

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Vitamin C/Ascorbic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25906

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.35 μg/mL

Detection range: 0.35-20 μg/mL

Average intra-assay CV (%): 2.3

Average inter-assay CV (%): 3.3

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used for detection of VC content in serum, plasma, animal/plant tissue samples.

▲Background

Vitamin C (ascorbic acid, VC) is a diacid with an enol structure that forms a five-membered heterocyclic lactone in organism. VC, as an enzyme cofactor for multiple enzymes, is used as an electron donor for monooxygenase and dioxygenase. Plants and most animals synthesize VC from glucose, but humans and non-human primates have lost the biosynthetic capacity of VC due to the loss of a functional mutation in the biosynthetic enzyme L-mannose-1,4-lactone oxidase.

▲Detection principle

The most obvious chemical activity of 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 vitamin C in plasma can be determined. Measure the OD value and calculate the VC content indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	10 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 2	Buffer Solution	50 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	12 mL ×1 vial	2-8℃ , 12 months, shading light
Reagent 4	Ferrum Reagent	1 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 5	Stop Solution	12 mL × 1 vial	2-8℃ , 12 months
Reagent 6	VC Standard	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.			

▲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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The sample pretreated with reagent 1 must be clarified after centrifugation.
2. Standard is easy to oxidized, it is best to use within 10 min.

Pre-assay preparation

▲ Reagent preparation

1. Preparation of reagent 1 application solution:

Dilute the reagent 1 with double distilled water at a ratio of 1:14 and mix fully. The prepared solution can be stored at 2-8℃ for 7 days.

2. Preparation of reagent 3 application solution:

Dilute the reagent 3 with absolute ethanol (self-prepared) at a ratio of 1:9 and mix fully. The prepared solution can be stored at 2-8℃ for 7 days with shading light.

3. Preparation of reagent 4 application solution:

Dilute 0.15 mL of reagent 4 with double distilled water to a final volume of 25 mL. Prepare the fresh solution before use.

4. Preparation of 6 μg/mL standard solution:

Dissolve a vial of reagent 6 with 1 mL of reagent 1 application solution to prepare 6 mg/mL standard solution. Then dilute 6 mg/mL standard solution with reagent 1 application solution for 1000 times to prepare 6 μg/mL standard solution.

Note: VC standard is easy to oxidized, it is best to use 6 μg/mL VC standard solution within 10 min.

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲ Sample requirements

The samples should not contain DTT, 2-mercaptoethanol and other reductive reagents. HEDP, EDTA and other chelating agents should not be added.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.35-20 μg/mL).

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).

Assay protocol

▲The preparation of of samples

Take 0.15 mL of sample, add 0.45 mL of reagent 1 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.

▲Detailed operation steps

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

(2) Add 0.5 mL of reagent 2, 1 mL of reagent 3 application solution and 0.25 mL of reagent 4 application solution to each tube.

(3) Mix fully with a vortex mixer and incubate at 37℃ water bath for 30 min.

(4) Add 0.1 mL of reagent 5 and mix fully 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.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Reagent 1 application solution (mL)	0.4		
6 μg/mL standard solution (mL)		0.4	
Supernatant (mL)			0.4
Reagent 2 (mL)	0. 5	0.5	0.5
Reagent 3 application solution (mL)	1	1	1
Reagent 4 application solution (mL)	0.25	0.25	0.25
Mix fully and incubate at 37°C water bath for 30 min.			
Reagent 5 (mL)	0.1	0.1	0.1
Mix fully and stand for 10 min at room temperature. Set the spectrophotometer to zero and measure the OD values of each tube.			

▲Calculation

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.

Appendix I Data

▲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 table.

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:

VC content(μg/mL) =(0.284 - 0.049)÷(0.256- 0.049)×6×4 = 27.2 μg/mL

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 3100 g at 4℃. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

Appendix III References

1. Loewus F A. Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. Phytochemistry, 1999, 52: 193–210.
2. Rumsey S C, Levine M. Absorption, transport, and disposition of ascorbic acid in humans. Journal of Nutritional Biochemistry, 1998, 9(3): 116–130.
3. Loewus F A, Loewus M W, Seib P A. Biosynthesis and metabolism of ascorbic acid in plants. Critical Reviews in Plant Sciences, 1987, 5(1): 101-119.
4. Du J, Cullen J J, Buettner G R. Ascorbic acid: chemistry, biology and the treatment of cancer. Biochimica et Biophysica Acta, 2012, 1826(2): 443-457.
5. Smirnoff N. Ascorbic acid metabolism and functions: A comparison of plants and mammals. Free Radical Biology & Medicine, 2018.