PRODUCT INFORMATION & MANUAL

Vitamin C/Ascorbic Acid Assay Kit (Colorimetric)

NBP3-25905

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Vitamin C/Ascorbic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25905

Method: Colorimetric method

Specification: 96T (Can detect 82 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.31 μg/mL

Detection range: 0.31-17.5 μg/mL

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 6.1

Average recovery rate (%): 108

- ▲ 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 measure 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 sample can be determined. Measure the OD value and calculate the VC content indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	5 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Buffer Solution	15 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	6 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 4	Ferrum Reagent	0.5 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 × 2 vials	2-8℃, 12 months, shading light
	Microplate	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.

▲Materials prepared by users

Instruments

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

Reagents

Double distilled water, Normal saline (0.9% NaCl), 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. 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.

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 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 the reagent 4 with double distilled water to a final volume of 25 mL. The prepared solution can be stored at 2-8 ℃ for 7 days with shading light.

4. Preparation of 60 μg/mL VC standard solution

   Dissolve a vial of reagent 6 with 1 mL of reagent 1 application solution to prepare 6 mg/mL VC standard solution. Then dilute 6 mg/mL VC standard solution with reagent 1 application solution for 100 times to prepare 60 μg/mL VC standard solution.

   Note: VC standard is easy to oxidized, it is best to use 60 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 (0.31-20 μg/mL).

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

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
B	B	B	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
C	C	C	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
D	D	D	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
E	E	E	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
F	F	F	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80
G	G	G	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81
H	S1	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82

Note: A-G, standard wells; S1-S82, sample wells.

▲Detailed operation steps

1. The pretreatment of sample supernatant

Take 0.10 mL of sample, add 0.30 mL of reagent 1 application solution, mix fully with vortex mixer and stand for 15 min at room temperature, then centrifuge at 2000 g for 10 min. Take the supernatant for test.

2. The preparation of standard curve

Dilute 60 μg/mL VC standard solution with reagent 1 application solution 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:

Number	Standard concentrations (μg/mL)	60 μg/mL VC standard solution (μL)	Reagent 1 (μL)
A	0	0	600
B	5.0	50	550
C	7.5	75	525
D	10.0	100	500
E	12.5	125	475
F	15.0	150	450
G	17.5	175	425

3. 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 reagent 2, 250 μL of reagent 3 application solution and 65 μL of reagent 4 application solution.
3) Mix fully with a vortex mixer and incubate at 37℃ for 30 min.
4) Add 25 μL of reagent 5 and mix fully 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.

▲Summary operation table

	Standard tube	Sample tube
Standard solution with different concentrations (μL)	100	
Sample supernatant (μL)		100
Reagent 2 (μL)	125	125
Reagent 3 application solution (μL)	250	250
Reagent 4 application solution (μL)	65	65
Mix fully and incubate at 37℃ for 30 min.		
Reagent 5 (μL)	25	25
Mix fully. Stand for 10 min at room temperature. Take 250 μL of reaction solution and measure the OD value.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.
The standard curve is: y= ax + b.

Serum/plasma samples:

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

Tissue samples:

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

Note:

y: $OD_{Standard} - OD_{Blank.}$

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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.

Appendix I Performance characteristics

▲Example analysis

Take 0.1 mL of chicken serum, add 0.30 mL of reagent 1 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 table. 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:

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

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 10000 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: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

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.
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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.