TECHNICAL MANUAL

Vitamin C (VC) Colorimetric Assay Kit

- **SKU CODE:** MAES0048
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Sample pretreatment
- Add reagents and incubate
- Measure OD value

2. Intended use

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

3. Detection principle

The most obvious chemical activity of Vitamin C (VC) is its ability to reduce Fe^{3+} to Fe^{2+}, which promotes iron absorption in the intestine and facilitates the storage and utilization of iron. Fe^{3+} reacts immediately with reducing ascorbic acid to form Fe^{2+}. Subsequently, Fe^{2+} reacts with phenanthroline, resulting in a color-developing reaction. The content of VC in the sample can be determined by measuring the OD value and calculating the VC content indirectly.

4. 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°C, 12 months, shading light
Reagent 2	Buffer Solution	8 mL × 1 vial	15 mL × 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Ferrum Reagent	0.5 mL × 1 vial	0.5 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 5	Stop Solution	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 6	VC Standard	6 mg × 2 vials	6 mg × 2 vials	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. 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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of extracting working solution:** Before testing, prepare sufficient extracting working solution according to the number of test wells. For example, to prepare 4.5 mL of extracting working solution, thoroughly mix 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. **Preparation of chromogenic working solution:** For each well, prepare 250 µL of chromogenic working solution by thoroughly mixing 25 µL of chromogenic agent with 225 µL of absolute ethanol. Store at 2-8°C for 7 days, protected from light.
4. **Preparation of ferrum working 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. **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. **Preparation of 60 µg/mL VC standard solution:** Before testing, prepare sufficient 60 µg/mL VC standard solution according to the number of test wells. For example, to prepare 700 µL of 60 µg/mL VC standard solution, thoroughly mix 7 µL of 6 mg/mL VC standard solution with 693 µL of extracting working solution. Note:

VC standard is easily oxidized; it is best to use the 60 μg/mL VC standard solution within 10 minutes.

7. **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 to create a serial concentration. The recommended dilution gradient is as follows: 0, 5, 7.5, 10, 12.5, 15, 17.5 μg/mL.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one 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°C.

4. Centrifuge at 10,000 × g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only): Most samples (human serum, mouse serum, chicken serum, horse serum, 10% tissue homogenates from various sources) require no dilution (dilution factor = 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 perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. VC standard is easily oxidized; please prepare freshly.
2. In the step of sample supernatant pretreatment, the supernatant after centrifugation should be clear.

9. Operating steps

1. **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 2,000 × g for 10 min. Take the supernatant for testing.

2. **Measurement of samples:** Standard wells: Add 100 µL of standard solution with different concentrations to 2 mL EP tubes. Sample wells: Add 100 µL of sample supernatant to 2 mL EP tubes.
3. Add 125 µL of buffer solution, 250 µL of chromogenic working solution, and 65 µL of ferrum working solution.
4. Mix well with a vortex mixer and incubate at 37°C for 30 min.
5. Add 25 µL of stop solution and mix well with a vortex mixer.
6. 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.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using the absolute OD value of standards and corresponding concentrations 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:

VC content (µg/mL) = (ΔA_536 - b) ÷ a × f × 4*

2. Tissue sample:

VC content (µg/mgprot) = (ΔA_536 - b) ÷ a × f × 4* ÷ C_{pr}

[Note]

f: Dilution factor of sample before testing.

ΔA_536: OD_{Sample} - OD_{Blank}.

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

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	1.50	8.40	12.50
%CV	5.8	6.4	6.1

Recovery

	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.

Standard curve

Concentration (µg/mL)	Average OD	Absolute OD
0	0.054	0
5	0.152	0.098
7.5	0.193	0.139

Concentration (μg/mL)	Average OD	Absolute OD
10	0.231	0.177
12.5	0.278	0.224
15	0.312	0.258
17.5	0.358	0.304

12. Appendix II Example Analysis

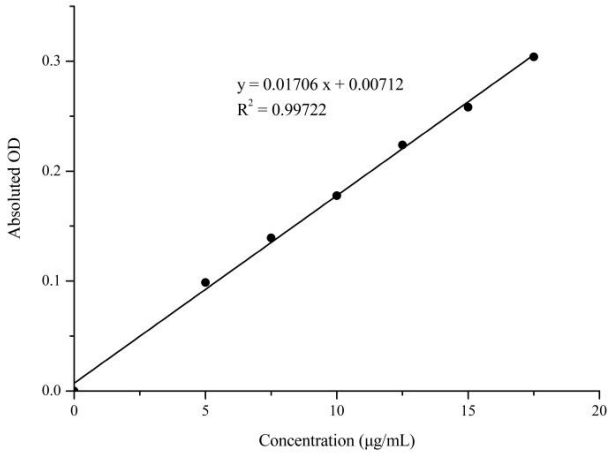
Example analysis:

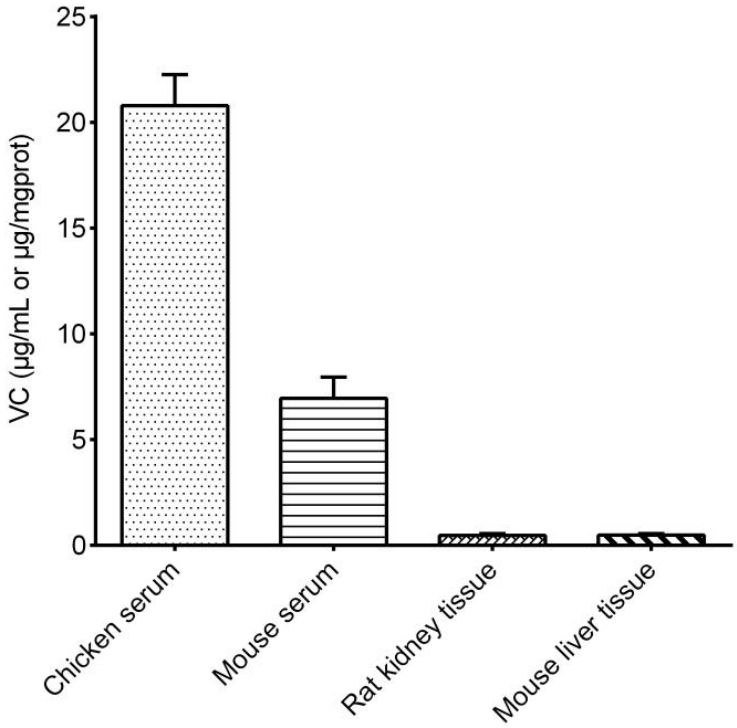
Take 0.1 mL of chicken serum, add 0.30 mL of extracting working solution, mix thoroughly with vortex mixer and stand for 15 min at room temperature, centrifuge at 2,000 × g for 10 min, then take the supernatant and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.0233x + 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:

VC content (μg/mL) = (0.203 - 0.062 - 0.0198) ÷ 0.0233 × 4 = 20.81 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 results are as follows:





13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.