TECHNICAL MANUAL

Vitamin E (VE) Colorimetric Assay Kit

- **SKU CODE:** MAES0046
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- n-heptane extraction
- Chromogenic reaction
- Measurement and calculation

2. Intended use

This kit can be used to measure Vitamin E content in serum, plasma and tissue samples.

3. Detection principle

Fe3+ can be reduced to Fe2+ by Vitamin E (VE) in the presence of ferroin. Fe2+ can react with phenanthroline and form a pink compound under certain conditions. After colorimetric assay, VE content can be determined according to the standard curve or calculated through the formula.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent	Powder × 1 vial	Powder × 1 vial	2-8 °C, 12 months, shading light
Reagent 2	Ferrum Reagent	Powder × 1 vial	Powder × 1 vial	2-8 °C, 12 months, shading light
Reagent 3	Stop Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8 °C, 12 months
Reagent 4	Homogenized Medium	50 mL × 1 vial	50 mL × 2 vials	2-8 °C, 12 months
Reagent 5	1 mg/mL VE Standard	0.4 mL × 1 vial	0.4 mL × 1 vial	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 (525-533 nm), Micropipettor, Centrifuge, Vortex mixer

Reagents:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of chromogenic application solution:** Dissolve one vial of chromogenic agent with 13 mL of absolute ethanol (self-prepared). Store at 2-8 °C for 7 days protected from light. This reagent is difficult to dissolve; it is recommended to prepare it 3-4 hours before use and ensure that the powder has been dissolved fully.
3. **Preparation of ferrum stock solution:** Dissolve one vial of ferrum reagent with 25 mL of absolute ethanol. Store at 2-8 °C for 7 days protected from light.
4. **Preparation of ferrum application solution:** For each well, prepare 15 µL of ferrum stock solution (mix well 1.5 µL of ferrum stock solution and 13.5 µL of absolute ethanol). The ferrum application solution should be prepared immediately before use.
5. **Preparation of 100 µg/mL standard solution:** Dissolve 72.5 µL of 1 mg/mL VE Standard with 652.5 µL of absolute ethanol. The 100 µg/mL standard solution should be prepared immediately before use.
6. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 µg/mL standard application solution with absolute ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 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 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 homogenized medium 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.

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

10% Mouse liver tissue homogenate - 1

10% Mouse brain tissue homogenate - 1

10% Rat kidney tissue homogenate - 1

10% Rat lung tissue homogenate - 1

10% Rat spleen tissue homogenate - 1

10% Rat heart tissue homogenate - 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. Test tubes should be cleaned with cleaning agent or boiling water, then washed with running water for second washing and double distilled water for third washing.
2. It is recommended to prepare the needed amount of fresh ferrum reagent before use.
3. The time of the extraction of VE (1 min) and the chromogenic reaction (5 min) should be accurate.
4. As this kit is a micro-determination method, the first absorbed liquid should be discarded each time when changing a pipette. The pipette should be vertical when adding sample or reagent and avoid touching the tube wall.
5. Be careful when extracting the n-heptane extraction solution. Do not mix the second layer (water and absolute alcohol) into it, or the OD value will be influenced.

6. Tubes for chromogenic reaction should be dry.
7. During the process of standing, the test tube must be sealed to reduce the volatilization of absolute ethanol and n-heptane in the system.

9. Operating steps

Extraction of n-heptane:

For serum (plasma) samples:

1. Standard tube: add 0.15 mL of double distilled water and 0.3 mL of standard solution with different concentrations to the 2 mL EP tubes.

Sample tube: add 0.15 mL of serum (plasma) and 0.3 mL of absolute ethanol to the 2 mL EP tubes.

2. Mix well with a vortex mixer for 20 s.

3. Add 0.5 mL of N-heptane into each tube and mix well with a vortex mixer for 1 min.

4. Centrifuge at 3,100 × g for 10 min, add 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

For tissue homogenate samples:

1. Standard tube: add 0.15 mL of double distilled water and 0.3 mL of standard solution with different concentrations to the 2 mL EP tubes.

Sample tube: add 0.15 mL of tissue homogenate and 0.3 mL of absolute ethanol to the 2 mL EP tubes.

Blank tube: add 0.15 mL of homogenized medium and 0.3 mL of absolute ethanol to the 2 mL EP tubes.

2. Mix well with a vortex mixer for 20 s.

3. Add 0.5 mL of N-heptane into each tube and mix well with a vortex mixer for 1 min.

4. Centrifuge at 3,100 × g for 10 min, add 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

Chromogenic reaction:

1. Add 200 µL of n-heptane VE extraction solution to corresponding EP tube.

2. Add 25 µL of chromogenic application solution and 15 µL of ferrum application solution to each tube.

3. Mix well with a vortex mixer and record time immediately. Stand for 5 min accurately at room temperature.

4. Add 15 µL of stop solution and mix well with a vortex mixer for 10 s.

5. Add 250 μL of absolute ethanol and mix fully with a vortex mixer.

6. Stand at room temperature for 2 min. Take 200 μL of supernatant to microplate and measure the OD value at 533 nm with microplate reader.

10. 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:

VE content (μg/mL) = (ΔA_533 − b) ÷ a × f × 2*

2. Tissue sample:

VE content (μg/g) = (ΔA_533 − b) ÷ a × f × 2* ÷ m/V

[Note]

ΔA_533: $OD_{Sample} - OD_{Blank}$. (For serum (plasma) sample, OD_{Blank} is the OD value of 0 μg/mL standard solution. For tissue sample, OD_{Blank} is the OD value of blank tube)

m: Weight of sample, g.

V: The volume of homogenized medium of tissue sample, mL.

2*: The volume of standard is 0.3 mL, the volume of sample is 0.15 mL, so the sample was concentrated twice.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	2.60	24.50	32.50
%CV	4.3	3.7	3.7

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	2.60	24.50	32.50
%CV	6.1	6.4	6.4

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration tested 6 times in parallel to get an average recovery rate of 97%.

Recovery

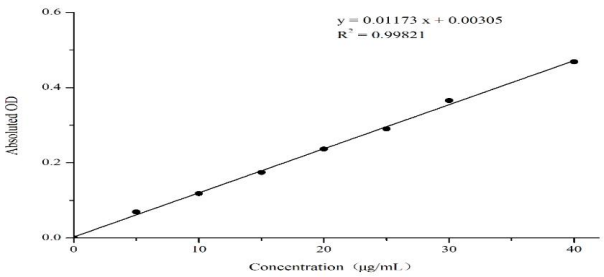
	Standard 1	Standard 2	Standard 3
Expected Conc. (µg/mL)	7.5	18	27.5
Observed Conc. (µg/mL)	7.1	17.8	26.7
Recovery rate (%)	95	99	97

Sensitivity

The analytical sensitivity of the assay is 0.95 µg/mL VE. 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

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), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (μg/mL)	Average OD	Absoluted OD
0	0.072	0
5	0.142	0.070
10	0.190	0.118
15	0.247	0.175
20	0.309	0.237
25	0.362	0.290
30	0.438	0.366
40	0.541	0.469

12. Appendix II Example Analysis

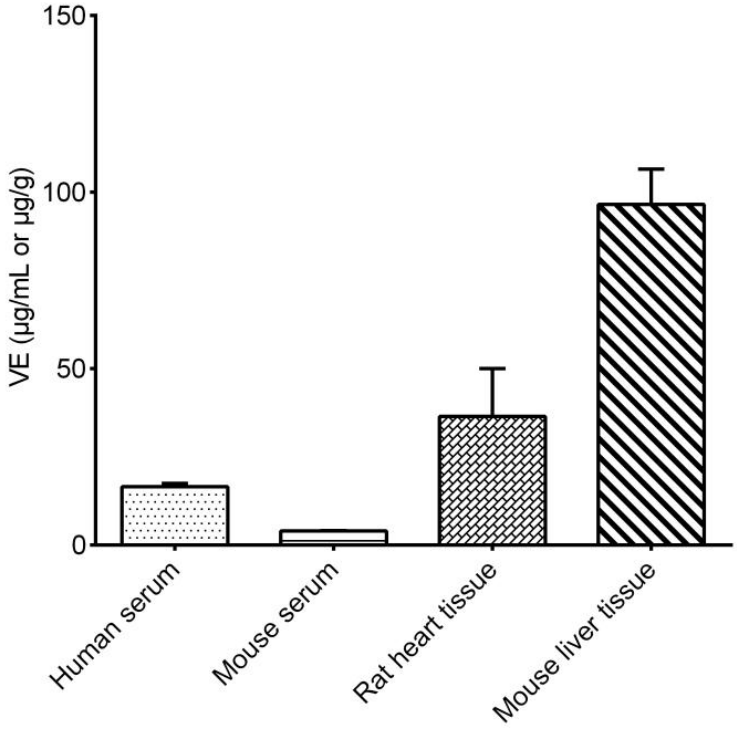
Example analysis:

Take 0.15 mL of human serum and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0094x + 0.0074, the average OD value of the sample is 0.152, the average OD value of the blank is 0.067, and the calculation result is:

VE content (μg/mL) = (0.152 - 0.067 - 0.0074) ÷ 0.0094 × 2 = 16.51 (μg/mL)

Detect human serum, mouse serum, 10% rat heart tissue homogenate and 10% mouse liver tissue homogenate 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.