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

Catalog No：E-BC-K033-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometer（533 nm）

Detection range：0.09-40 μg/mL

Elabscience® Vitamin E (VE) Colorimetric Assay Kit

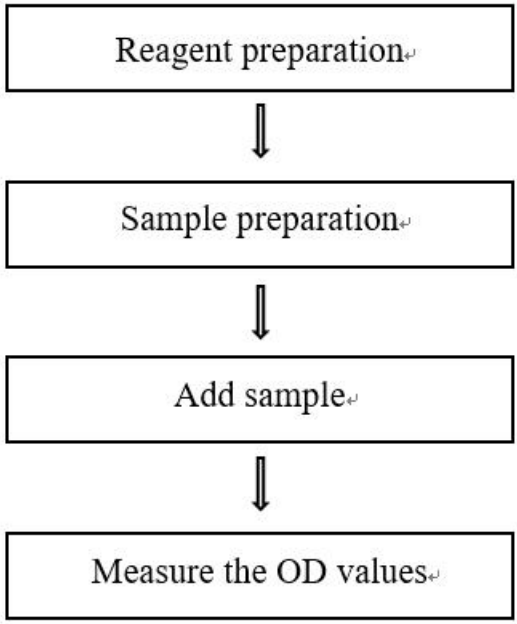
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

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

Detection principle

Fe^{3+} can be deoxidized to Fe^{2+} by Vitamin E (VE) with ferroin existing. Fe^{2+} can react with phenanthroline and form pink compound under certain condition. VE content can be calculated by measuring the OD value at 532 nm.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Chromogenic Agent	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
Reagent 2	Ferrum Reagent	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
Reagent 3	Stop Solution	6 mL × 1 vial	6 mL × 1 vial	2-8℃, 12 months
Reagent 4	Homogenized Medium	50 mL × 2 vials	50 mL × 4 vials	2-8℃, 12 months
Reagent 5	1 mg/mL VE Standard	0.4 mL × 1 vial	0.4 mL × 1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (533 nm), Micropipettor, Vortex mixer, Centrifuge

Reagents:

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

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of chromogenic application solution:
Dissolve one vial of chromogenic with 13 mL of absolute ethanol (self-prepared). Store protected from light. This reagent is difficult to be dissolved, it is recommended to prepare it 3~4 hours before use and make sure that the powder has been dissolved fully.

③ The preparation of ferrum stock solution:
Dissolve one vial of ferrum reagent with 25 mL of absolute ethanol. Store at 2-8°C protected from light.

④ The preparation of ferrum application solution:
For each well, prepare 50 μL of ferrum stock solution (mix well 5 μL of ferrum stock solution and 45 μL of absolute ethanol). Store at 2-8°C for 2 days protected from light.

⑤ The preparation of 10 μg/mL standard application solution:
For each well, prepare 600 μL of 10 μg/mL standard application solution (mix well 6 μL of 1 mg/mL VE Standard and 594 μL of absolute ethanol).

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 homogenized medium 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.

② Dilution of sample

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 liver tissue homogenization	1
Rat serum	1
Rat plasma	1
10% Carrot tissue homogenization	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 Test tubes should be cleaned with cleaning agent or boiling water, then wash with running water for second washing and double distilled water for third washing.

② It is recommended to prepare 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 changing a pipette. The pipette should be vertical when adding sample or reagent and avoid of 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.

Operating steps

Extraction of n-heptane:

For serum (plasma) samples

① Blank tube: add 0.3 mL of double distilled water, 0.6 mL of absolute ethanol into 5 mL EP tube.

Standard tube: add 0.3 mL of double distilled water, 0.6 mL of 10 μg/mL standard into 5 mL EP tube.

Sample tube: add 0.3 mL of serum (plasma), 0.6 mL of absolute ethanol into 5 mL EP tube.

② Mix well with a vortex mixer for 20 s.

③ Add 1.0 mL of N-heptane into each tube and mix well with a vortex mixer for 1 min.

④ Centrifuge at 3100×g for 10 min, add 0.8 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

Chromogenic reaction:

① Add 0.8 mL of n-heptane VE extraction solution to corresponding EP tube.

② Add 0.1 mL of chromogenic application solution and 0.05 mL 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 0.05 mL of stop solution and mix well with a vortex mixer for 10 s.

⑤ Add 1 mL of absolute ethanol and mix fully with a vortex mixer.

⑥ Stand at room temperature for 2 min. Set the spectrophotometer to zero with absolute ethanol and measure the OD values of each tube at 533 nm with of 0.5 cm optical path cuvette.

Extraction of n-heptane:

For tissue homogenate samples:

① Blank tube: Take 0.3 mL of double distilled water, 0.6 mL of absolute ethanol into 5 mL EP tube.

Standard tube: add 0.3 mL of double distilled water, 0.6 mL of 10 μg/mL standard into 5 mL EP tube.

Sample blank tube: add 0.3 mL of homogenized medium, 0.6 mL of absolute ethanol into 5 mL EP tube.

Sample tube: add 0.3 mL of tissue homogenate, 0.6 mL of absolute ethanol into 5 mL EP tube.

② Mix well with a vortex mixer for 20 s.

③ Add 1.0 mL of N-heptane into each tube and mix well with a vortex mixer for 1 min.

④ Centrifuge at 3100 g for 10 min, add 0.8 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

Chromogenic reaction:

① Add 0.8 mL of n-heptane VE extraction solution to corresponding EP tube.

② Add 0.1 mL of chromogenic application solution and 0.05 mL 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 0.05 mL of stop solution and mix well with a vortex mixer for 10 s.

⑤ Add 1 mL of absolute ethanol and mix fully with a vortex mixer.

⑥ Stand at room temperature for 2 min. Set the spectrophotometer to zero with absolute ethanol and measure the OD values of each tube at 533 nm with of 0.5 cm optical path cuvette.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{VE content } (\mu g/mL) = \frac{\Delta A_1}{\Delta A_2} \times c \times f \times 2^*$$

2. Tissue sample:

$$\text{VE content } (\mu g/g) = \frac{\Delta A_3}{\Delta A_2} \times f \times 2^* \div \frac{m}{V}$$

[Note]

ΔA_1: OD_{Sample} - OD_{Blank}

ΔA_2: $OD_{Standard}$ - OD_{Blank}

ΔA_3: OD_{Sample} - $OD_{Sample\ blank}$

c: Concentration of standard, 10 μg/mL

m: Weight of sample, g

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

f: Dilution factor of sample before test.

2*: The volume of standard is 0.6 mL, the volume of sample is 0.3 mL, so the sample was condensed twice.

.

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	18.50	35.00
%CV	4.7	4.3	4.2

Intra-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	18.50	35.00
%CV	5.2	4.7	4.8

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 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μg/mL)	0.1	0.3	0.5
Observed Conc. (μg/mL)	0.1	0.299	0.477
Recovery rate (%)	99	99	95

Sensitivity

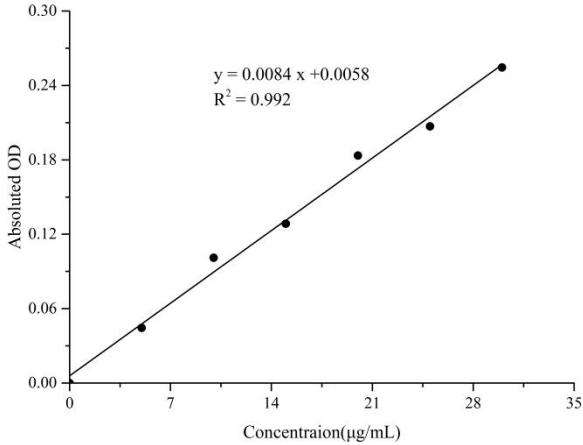
The analytical sensitivity of the assay is 0.09 μg/mL VE. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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	10	15	20	25	30
Average OD	0.029	0.074	0.130	0.158	0.213	0.236	0.284
Absoluted OD	0	0.045	0.101	0.129	0.184	0.207	0.255



Appendix II Example Analysis

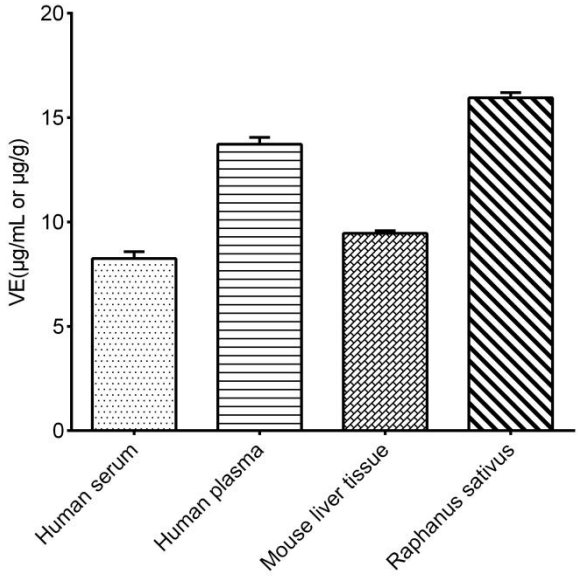
Example analysis:

Take 0.3 mL of human serum, carry the assay according to the operation steps. The results are as follows:

the average OD value of the sample is 0.128, the average OD value of the blank is 0.066, the average OD value of the standard is 0.157, and the calculation result is:

$$\text{VE content (μg/mL)} = \frac{(0.128 - 0.066)}{(0.157 - 0.066)} \times 10 \times 2 = 13.72 \text{ (μg/mL)}$$

Detect human serum, human plasma, 10% mouse liver tissue homogenate, 10% raphanus sativus tissue homogenate 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.