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

Catalog No：E-BC-K033-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (525-533 nm)

Detection range：0.95-40 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.

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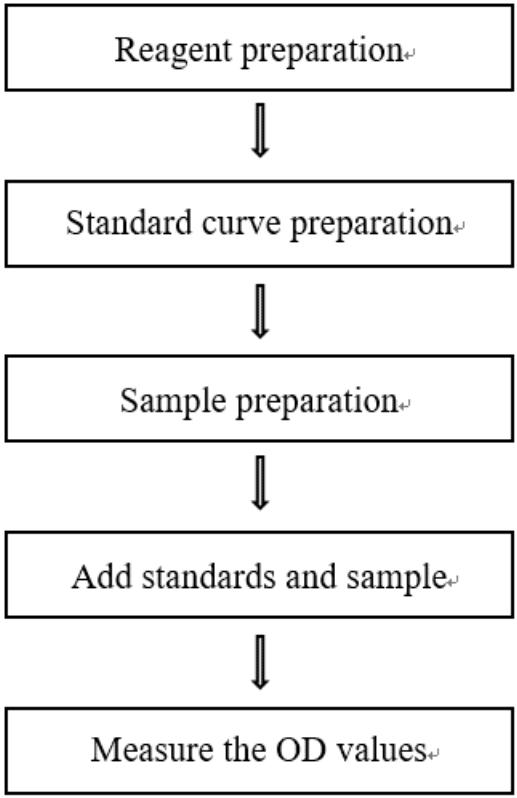
Assay summary



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. After colorimetric assay, VE content can be figured out according to the standard curve or calculated through formula.

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℃, 12 months shading light
Reagent 2	Ferrum Reagent	Powder ×1 vial	Powder ×1 vial	2-8℃, 12 months shading light
Reagent 3	Stop Solution	1.5 mL ×1 vial	1.5 mL ×2 vials	2-8℃, 12 months
Reagent 4	Homogenized Medium	50 mL ×1 vial	50 mL ×2 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
	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

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

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 at 2-8 ℃ for 7 days 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 ℃ for 7 days protected from light.

④ The preparation of ferrum application solution:

For each well, prepare 15 uL of ferrum stock solution (mix well 1.5 uL of ferrum stock solution and 13.5 uL of absolute ethanol). The ferrum application solution should be prepared on spot.

⑤ The 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 on spot.

⑥ The 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 diluent

to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 40 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**40**
100 μg/mL standard (μL)	0	25	50	75	100	125	150	200
Absolute ethanol (μL)	500	475	450	425	400	375	350	300

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
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 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 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 3100 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 3100 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.

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:

$$\text{VE content } (\mu g/mL) = (\Delta A_{533} - b) \div a \times f \times 2^{*}$$

2. Tissue sample:

$$\text{VE content } (\mu g/g) = (\Delta A_{533} - b) \div a \times f \times 2^{*} \div \frac{m}{V}$$

[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 condensed twice.

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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)	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.

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

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 97%.

	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

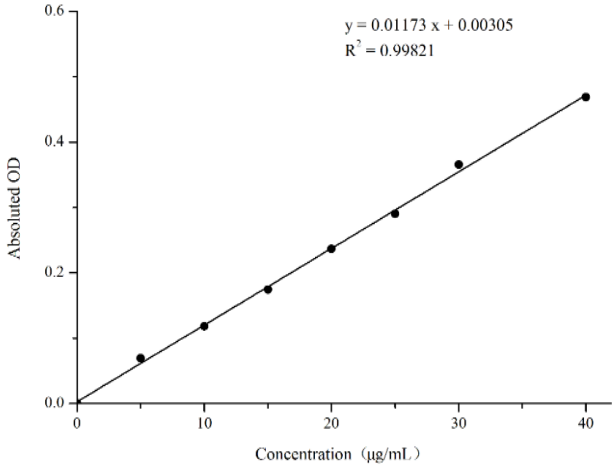
Sensitivity

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.

2. 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), so the standard curve and data are provided as below for reference only:

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



Appendix II Example Analysis

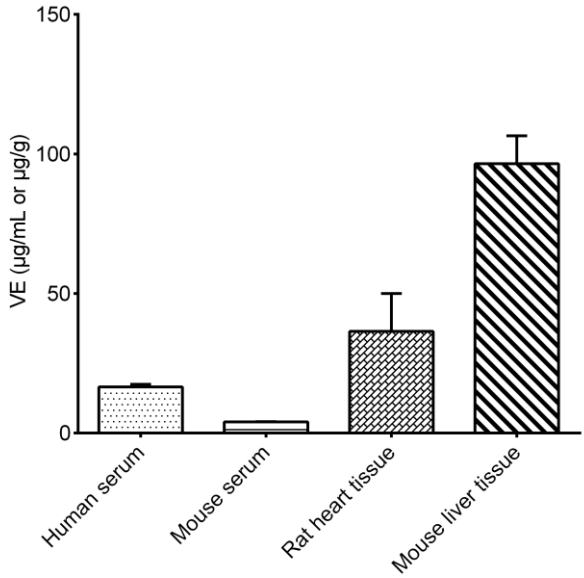
Example analysis：

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

standard curve: y = 0.0094 x + 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:

$$\text{VE content (μg/mL)} = (0.152 - 0.067 - 0.0074) \div 0.0094 \times 2 = 16.51\ (\mu g/mL)$$

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