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Vitamin E (VE) Assay Kit

Catalog No.: BC00035

Size: 100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

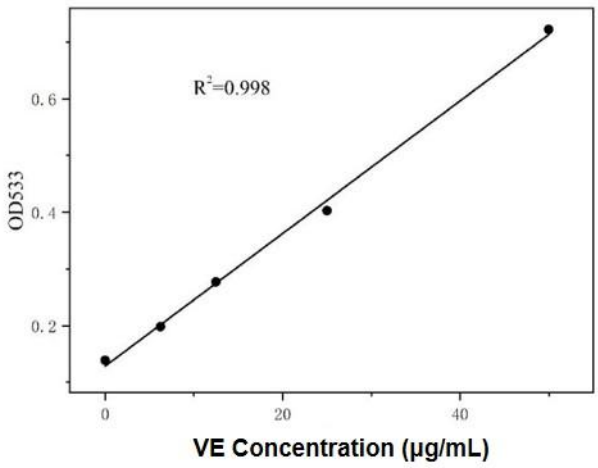
Product Name	Vitamin E (VE) Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma, animal and plant tissues
Detection Type	Quantitative
Detection instrument and wavelength	Microplate reader (533 nm)
Range	6.25-50μg/mL
Sensitivity	1μg/mL

Product Introduction

Vitamin E is a fat-soluble vitamin, and its hydrolysis product is tocopherol, which is one of the most important antioxidants in organisms. It can prevent unsaturated fatty acids from being damaged by peroxidation, maintain the integrity and normal function of unsaturated fatty acid cell membranes, and has the effects of delaying aging and preventing hemolytic anemia.

Detection Principle

In the presence of phenanthroline, vitamin E can reduce trivalent iron ions to divalent iron ions, which can form a pink complex with phenanthroline under certain conditions, with a maximum absorption wavelength at 533 nm. The content of vitamin E can be calculated by colorimetry.



Product Composition

Serial Number	Product Name	Packing Specifications (100T)	Storage

Reagent 1	Color developer	Powder × 1	store at 2-8℃ after opening
Reagent 2	Iron reagent	Powder × 1	store at 2-8℃ after opening
Reagent 3	Terminator	0.75ml×1	store at 2-8℃ after opening
Reagent 4	Homogenization medium	100ml	store at 2-8℃ after opening
Reagent 5	VE Standard	0.4mL	store at 2-8℃ after opening
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage conditions

The unopened kit can be stored at -20℃ for 12 months. After opening, it can be stored at 2-8 ℃ for 6 months.

Preparation before the experiment

Sample processing

1. Sample preparation

(1) Sample requirements: The sample cannot contain reducing reagents such as DTT and 2-mercaptoethanol, and cannot contain chelating agents such as HEDP and EDTA .

Serum (plasma) samples: can be measured directly.

Tissue samples: routine homogenization treatment (homogenization medium is reagent 4).

(2) Sample dilution

Before formal testing, it is necessary to select 2-3 samples with large expected differences and dilute them into different concentrations for preliminary experiments. According to the results of the preliminary experiments and the linear range of this kit: 1-50 μg/mL, please refer to the following table for dilution (for reference only):

sample	Dilution multiple	sample	Dilution multiple
Human serum	No dilution	10% rat kidney tissue	No dilution
Mouse serum	No dilution	10% rat lung tissue	No dilution
Chicken serum	No dilution	10% rat spleen tissue	No dilution
10% mouse liver	No dilution	10% rat heart tissue	No dilution
10% mouse brain	No dilution		

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

2 . Extraction of VE from samples

(1) Extraction of VE from serum (plasma) using n-heptane

a. Standard tube: Take 0.15 mL double distilled water and 0.3 mL of different concentrations of standard substances and add them into 2 mL EP tubes;

Assay tube: Take 0.15 mL of serum (plasma) and 0.3 mL of anhydrous ethanol in turn and add them into a 2 mL EP tube.

b. Vortex to mix for 20 s.

c. Add 0.5 mL of n-heptane to each tube in step b and vortex to mix for 1 min.

d. Centrifuge at 3000-4000rpm for 10 min, aspirate 0.2 mL of the upper n-heptane VE extract and perform color development reaction.

e . The operation table is as follows:

	Standard tube	**Determination tube**
Double distilled water (mL)	0.15	
Different concentrations of standard products (mL)	0.3	
Serum (plasma) (mL)		0.15
Anhydrous ethanol (mL)		0.3
Vortex mix for 20 s (protein precipitation)		
n-Heptane (mL)	0.5	0.5
Vortex mix (fully extract) for 1 min, centrifuge at 300-4000rpm for 10 min, and draw 0.2 mL of the upper n-heptane VE extract for color development.		

(2) Extraction of VE from tissue homogenate using n-heptane

a. Standard tube: Take 0.15 mL double distilled water and 0.3 mL of different concentrations of standard substances and add them into 2 mL EP tubes;

Determine the blank tube: take 0.15 mL of reagent IV and 0.3 mL of anhydrous ethanol, add them into a 2 mL EP tube ;

Assay tube: Take 0.15 mL of tissue homogenate and 0.3 mL of anhydrous ethanol and add them into a 2 mL EP tube .

b. Vortex to mix for 20 s.

c. Add 0.5 mL of n-heptane to each tube in step b and vortex to mix for 1 min.

d. Centrifuge at 3000-4000rpm for 10 min, aspirate 0.2 mL of the upper n-heptane VE extract and

perform color development reaction.

e. The operation table is as follows:

	Standard tube	Determination of blank tube	Determination tube
Double distilled water (mL)	0.15		
Different concentrations of	0.3		
Tissue homogenate (mL)			0.15
Reagent 4 (mL)		0.15	
Anhydrous ethanol (mL)		0.3	0.3
Vortex mix for 20 s (protein precipitation)			
n-Heptane (mL)	0.5	0.5	0.5
Vortex mix (fully extract) for 1 min, centrifuge at 3 000-4000 rpm for 10 min, and draw 0.2 mL of the upper n-heptane VE extract for color development.			

· Preparation of the kit

1. Before testing, the reagents in the kit were equilibrated to room temperature.
2. Preparation of reagent 1 working solution: Take reagent 1 powder and dissolve it with 1.25 mL of anhydrous ethanol by shaking. It can be stored at 2-8 ℃ and away from light for 7 days. (This powder is difficult to dissolve and needs to be prepared 3-4 hours in advance).
3. Preparation of reagent 2 working solution: Dissolve the reagent 2 powder in 0.75 mL of anhydrous ethanol and store at 2-8°C away from light for 7 days.
4. Preparation of 100 μg/mL standard: Take an appropriate amount of reagent 5, dilute it 10 times with anhydrous ethanol, mix well, and use it immediately after preparation.
5. Dilution of different concentrations of standard samples: Use the half-dilution method to dilute the standard sample with anhydrous ethanol to 50, 25, 12.5, 6.25, and 0 (blank well) μg/mL.

Operation process

1. Take 100 μL of VE n-heptane extract from each tube and add it to the ELISA plate.
2. To each well in step (1), add 12.5 μL of Reagent 1 working solution and 7.5 μL of Reagent 2

working solution.

3. Start timing immediately and let stand at room temperature for 5 min.

4. Add 7.5 μL of reagent 3 to each tube in step (3).

5. Add 100 μL of anhydrous ethanol to each tube in step (4).

6. After standing at room temperature for 2 min, the OD value of each well was measured at 533 nm using an ELISA reader.

The operation table is as follows:

	Standard well	**Determination of blank wells**	**Determination well**
n-Heptane VE extract (μL)	100	100	100
Reagent **1** working solution (μL)	12.5	12.5	12.5
Reagent 2 working solution (μL)	7.5	7.5	7.5
Record the time immediately and let it stand at room temperature for 5 min .			
Reagent 3 (μL)	7.5	7.5	7.5
Anhydrous ethanol (μL)	100	100	100
After standing at room temperature for 2 min, the OD value of each well was measured at 533 nm using an ELISA reader			

Result calculation

Standard fitting curve: y = ax + b

1. The formula for calculating the VE content in serum (plasma): VE content = (ΔA_{533}-b) ÷ a × f × 2* (μg/mL)

2. Calculation formula of VE content in tissue: VE content = (ΔA533-b) ÷ a × f × 2* × m/V (μg/g)

annotation:

y: OD value of standard sample minus blank OD value

x: concentration corresponding to the absorbance

a: slope of the curve

b: intercept of the curve

ΔA_{533}: Sample measured OD value - blank OD value (serum (plasma) sample refers to the OD value of the standard concentration of 0; tissue sample refers to the measured blank OD value)

f: dilution factor of the sample before adding it to the detection system

m: tissue sample weight (g)

V: Volume of reagent IV (homogenization medium) added during tissue sample processing (mL)
2*: In the sample extraction step, the amount of standard added is 0.3 mL, and the amount of sample added is 0.15 mL, so ×2 is required for VE calculation.

Notes

1. The color development reaction between reagent 1 and reagent 2 should be accurate for 5 minutes.
2. The extraction time of vitamin E (1 minute) must be sufficient.
3. When absorbing the n-heptane extract, be sure to absorb it carefully and do not mix the second layer (i.e. the liquid phase layer of water and anhydrous ethanol), otherwise it will affect the absorbance value.
4. This product is limited to scientific research by professionals and shall not be used for clinical diagnosis or treatment, shall not be used as food or medicine, and shall not be stored in ordinary residences.