

## Vitamin E (VE) Colorimetric Assay Kit

Catalog No: E-BC-K033-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.95  $\mu\text{g/mL}$

Detection range: 0.95-40  $\mu\text{g/mL}$

- ▶ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## General information

### ▲ Intended use

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

### ▲ Background

Vitamin E is a fat-soluble antioxidant that blocks the development of ester peroxidation chain reactions to maintain the integrity of cell membranes. The term of vitamin E includes four tocopherols and four tocotrienols, of which  $\alpha$ -tocopherol is the most biologically active form of vitamin E.

### ▲ Detection principle

$\text{Fe}^{3+}$  can be deoxidized to  $\text{Fe}^{2+}$  by VE with ferroin existing.  $\text{Fe}^{2+}$  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	Specification	Storage
Reagent 1	Chromogenic Agent	Powder × 1 vial	2-8 °C, 6 months, shading light
Reagent 2	Ferrum Reagent	Powder × 1 vial	2-8 °C, 6 months, shading light
Reagent 3	Stop Solution	1.5 mL × 2 vials	2-8 °C, 6 months
Reagent 4	Homogenized Medium	50 mL × 2 vials	2-8 °C, 6 months
Reagent 5	1 mg/mL VE Standard	0.4 mL × 1 vial	2-8 °C, 6 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
<p>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.</p>			

## ▲ Materials prepared by users



### Instruments

Microplate reader (525-533 nm), Micropipettor, Centrifuge, Vortex mixer



### Consumptive material

Tips (10  $\mu$ L, 200  $\mu$ L, 1000  $\mu$ L), EP tubes (1.5 mL, 2 mL, 10 mL)



### Reagents

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

## ▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

## ▲ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ 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 reagent 2 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.

## Pre-assay preparation

### ▲ Reagent preparation

#### 1. Preparation of reagent 1 application solution

Dissolve 1 vial of reagent 1 powder with 13 mL of absolute ethanol (self-prepared). The prepared solution can be stored at 2-8 °C for 7 days with shading 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.

#### 2. Preparation of reagent 2 stock solution

Dissolve 1 vial of reagent 2 powder with 25 mL of absolute ethanol. The prepared solution can be stored at 2-8 °C for 7 days with shading light.

#### 3. Preparation of reagent 2 application solution

Dilute the reagent 2 stock solution for 10 times with absolute ethanol. Prepare the fresh solution before use.

#### 4. Preparation of 100 µg/mL standard application solution

Dilute the reagent 5 with absolute ethanol for 10 times. Prepare the fresh solution before use.

### ▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

#### Sample requirements

The sample should not contain reducing reagents such as DTT and 2-mercaptoethanol, and no chelating agents such as HEDP and EDTA.

### ▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.95-40 µg/mL).

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).

Assay protocol	
Ambient temperature	25-30
Optimum detection wavelength	533 nm

### Instructions for the use of transferpette

- (1) Equilibrate the pipette tip in that reagent before pipetting each reagent.
- (2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

## Assay protocol

### ▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.



## ▲ Operating steps

### 1. The preparation of standard curve

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.

### 2. Extraction of n-heptane

#### 1) For serum (plasma) samples

a) **Standard tube:** Take 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:** Take 0.15 mL of serum (plasma) and 0.3 mL of absolute ethanol to the 2 mL EP tubes.

b) Mix fully with a vortex mixer for 20 s.

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

d) Centrifuge at 3100 g for 10 min, take 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

## 2) For tissue homogenate samples

a) **Standard tube:** Take 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:** Take 0.15 mL of tissue homogenate and 0.3 mL of absolute ethanol to the 2 mL EP tubes.

**Blank tube:** Take 0.15 mL of reagent 4 and 0.3 mL of absolute ethanol to the 2 mL EP tubes.

b) Mix fully with a vortex mixer for 20 s.

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

d) Centrifuge at 3100 g for 10 min, take 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

## 3. Chromogenic reaction

1) Take 200  $\mu$ L of n-heptane VE extraction solution to corresponding EP tube.

2) Add 25  $\mu$ L of reagent 1 application solution and 15  $\mu$ L of reagent 2 application solution to each tube.

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

4) Add 15  $\mu$ L of reagent 3 and mix fully with a vortex mixer for 10 s.

5) Add 250  $\mu$ L of absolute ethanol and mix fully with a vortex mixer.

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

**Note:** For serum (plasma) sample, the blank tube is not required.

## ▲ Operation table

### 1. Extraction of n-heptane

#### 1) For serum (plasma) samples

	Standard tube	Sample tube
Double distilled water(mL)	0.15	
Standard solution with different concentrations (mL)	0.3	
Serum (plasma) (mL)		0.15
Absolute ethanol (mL)		0.3
Mix fully with a vortex mixer for 20 s (protein precipitation).		
N-heptane(mL)	0.5	0.5
Mix fully with a vortex mixer for 1 min, then centrifuge at 3100 g for 10 min, take 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.		

#### 2) For tissue homogenate samples

	Standard tube	Sample tube	Blank tube
Double distilled water (mL)	0.15		
Standard solution with different concentrations (mL)	0.3		
Tissue homogenate (mL)		0.15	
Reagent 4 (mL)			0.15
Absolute ethanol (mL)		0.3	0.3
Mix fully with a vortex mixer for 20 s (protein precipitation).			
N-heptane(mL)	0.5	0.5	0.5
Mix fully with a vortex mixer for 1 min, then centrifuge at 3100 g for 10 min, take 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.			

## 2. Chromogenic reaction

	Standard tube	Blank tube	Sample tube
N-heptane VE extraction solution ( $\mu\text{L}$ )	200	200	200
Reagent 1 application solution ( $\mu\text{L}$ )	25	25	25
Reagent 2 application solution ( $\mu\text{L}$ )	15	15	15
Mix fully and record time immediately, stand for 5 min accurately at room temperature.			
Reagent 3 ( $\mu\text{L}$ )	15	15	15
Mix fully with a vortex mixer for 10 s.			
Absolute ethanol ( $\mu\text{L}$ )	250	250	250
Mix fully with a vortex mixer and stand at room temperature for 2 min. Take 200 $\mu\text{L}$ of supernatant to microplate and measure the OD value at 533 nm with microplate reader.			

**Note:** For serum (plasma) sample, the blank tube is not required.

### ▲ Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is:  $y = ax + b$ .

#### 1. Serum/plasma samples:

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

#### 2. Tissue samples:

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

#### Note:

y:  $OD_{\text{Standard}} - OD_{\text{Blank}}$  ( $OD_{\text{Blank}}$  is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

$\Delta A_{533}$ :  $OD_{\text{Sample}} - OD_{\text{Blank}}$  (For serum (plasma) sample,  $OD_{\text{Blank}}$  is the OD value of 0  $\mu\text{g/mL}$  standard solution. For tissue sample,  $OD_{\text{Blank}}$  is the OD value of blank tube)

m: Weight of sample, g.

V: The volume of homogenized medium (reagent 4) 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.

## ▲ Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

## Appendix I Performance characteristics

Appendix I Performance characteristics			
Detection range	0.95-40 µg/mL	Average intra-assay CV (%)	3.9
Sensitivity	0.95 µg/mL	Average inter-assay CV (%)	6.3
Average recovery rate (%)	97		

### ▲ Example analysis

Take 0.15 mL of human serum and carry the assay according to the operation table. 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:

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

## Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

### ▲ Serum

Collect fresh blood and stand at 25 °C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4 °C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80 °C for a month.

### ▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4 °C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80 °C for a month.

### ▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2 - 8 °C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2 - 8 mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000g at 4 °C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80 °C for a month.



**Note:**

1. Homogenized medium: Reagent 4.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces ( $1\text{ mm}^3$ ), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

▲ **Note for sample**

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.