Vitamin E (VE) Colorimetric Assay Kit

Catalog No: E-BC-K033-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.09 μg/mL

Detection range: 0.09-40 μg/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

VE is a kind of natural lipid-soluble antioxidant, which exist in cellular membrane structure (cell membrane, mitochondrial, microsomal membrane), lipid droplets of adipocytes and lipoproteins of plasma. It is scavenger of singlet oxygen and superoxide, blocking agent of lipid peroxidation and can protect protein sulfhydryl.

▲Detection principle

Fe^{3+} can be deoxidized to Fe^{2+} by 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 533 nm.

▲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	6 mL × 1 vial	2-8°C , 6 months
Reagent 4	Homogenized Medium	50 mL × 4 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
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.			

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL, 10 mL)

Reagents:

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 in 13 mL of absolute ethyl alcohol (self-prepared). Store the solution in dark. 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 in 25 mL of absolute ethyl alcohol. Store the stock solution in dark.
3. Preparation of reagent 2 application solution
   Dilute the reagent 2 stock solution for 10 times with absolute ethyl alcohol. The prepared Reagent 2 application solution can be stored at 2~8°C in dark within 2 days.
4. Preparation of 10 μg/mL standard application solution
   Dilute the 1 mg/mL standard stock solution for 100 times with absolute ethyl alcohol.

▲ Sample preparation

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

▲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 (0.09-40 μg/mL).

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).

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

Instructions for the use of transferpettor:

(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

▲Operating steps

For serum (plasma) samples

1. Extraction of n-heptane

(1) Blank tube: Take 0.3 mL of double distilled water, 0.6 mL of absolute ethanol into 5 mL EP tube.
Standard tube: Take 0.3 mL of double distilled water, 0.6 mL of 10 μg/mL standard into 5 mL EP tube.
Sample tube: Take 0.3 mL of serum (plasma), 0.6 mL of absolute ethanol into 5 mL EP tube.

(2) Mix fully with a vortex mixer for 20 s.

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

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

2. Chromogenic reaction

(1) Take 0.8 mL of n-heptane VE extraction solution into each tube of EP tube.

(2) Add 0.1 mL of reagent 1 application solution and 0.05 mL of reagent 2 application solution into each tube.

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

(4) Add 0.05 mL of reagent 3 and mix fully 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 absolute ethanol and measure the OD values of each tube at 533 nm with of 0.5 cm optical path cuvette.

For tissue homogenate samples

1. Extraction of n-heptane

(1) Blank tube: Take 0.3 mL of double distilled water, 0.6 mL of absolute ethanol into 5 mL EP tube.
Standard tube: Take 0.3 mL of double distilled water, 0.6 mL of 10 μg/mL standard into 5 mL EP tube.
Control tube: Take 0.3 mL of reagent 4, 0.6 mL of absolute ethanol into 5 mL EP tube.
Sample tube: Take 0.3 mL of tissue homogenate, 0.6 mL of absolute ethanol into 5 mL EP tube.

(2) Mix fully with a vortex mixer for 20 s.

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

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

2. Chromogenic reaction

(1) Take 0.8 mL of n-heptane VE extraction solution into each tube of EP tube.

(2) Add 0.1 mL of reagent 1 application solution and 0.05 mL of reagent 2 application solution into each tube.

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

(4) Add 0.05 mL of reagent 3 and mix fully 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 absolute ethanol and measure the OD values of each tube at 533 nm with of 0.5 cm optical path cuvette.

▲Operation table

For serum (plasma) samples

1. Extraction of n-heptane

	Blank tube	Standard tube	Sample tube
Sample (mL)			0.3
Double-distilled water(mL)	0.3	0.3	
10 μg/mL VE standard solution(mL)		0.6	
Absolute ethanol (mL)	0.6		0.6
Mix fully with a vortex mixer for 20 s (protein precipitation).			
N-heptane (mL)	1.0	1.0	1.0
Mix fully with a vortex mixer for 1 min, centrifuge at 3100 g for 10 min, take 0.8 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.			

2. Chromogenic reaction

	Blank tube	Standard tube	Sample tube
N-heptane VE extraction solution (mL)	0.8	0.8	0.8
Reagent 1 application solution (mL)	0.1	0.1	0.1
Reagent 2 application solution (mL)	0.05	0.05	0.05
Mix fully and record time immediately, stand for 5 min accurately.			
Reagent 3 (mL)	0.05	0.05	0.05
Mix fully with a vortex mixer for 10 s.			
Absolute ethanol (mL)	1.0	1.0	1.0
Mix fully with a vortex mixer and 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.			

For tissue homogenate samples

1. Extraction of n-heptane

	Blank tube	Control tube	Standard tube	Sample tube
10% homogenate supernatant (mL)				0.3
Double-distilled water (mL)	0.3		0.3	
Reagent 4		0.3		
10 μg/mL VE standard solution (mL)			0.6	
Absolute ethanol (mL)	0.6	0.6		0.6
Mix fully with a vortex mixer for 20 s (protein precipitation).				
N-heptane (mL)	1.0	1.0	1.0	1.0
Mix fully with a vortex mixer for 1 min, centrifuge at 3100 g for 10 min, take 0.8 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.				

2. Chromogenic reaction

	Blank tube	Control tube	Standard tube	Sample tube
N-heptane VE extraction solution (mL)	0.8	0.8	0.8	0.8
Reagent 1 application solution (mL)	0.1	0.1	0.1	0.1
Reagent 2 application solution (mL)	0.05	0.05	0.05	0.05
Mix fully and record time immediately, stand for 5 min accurately.				
Reagent 3 (mL)	0.05	0.05	0.05	0.05
Mix fully with a vortex mixer for 10 s.				
Absolute ethanol (mL)	1.0	1.0	1.0	1.0
Mix fully with a vortex mixer and 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

1. Serum (plasma) sample:

$$VE\ (\mu g/mL) = \frac{OD_{sample}-OD_{blank}}{OD_{standard}-OD_{blank}} \times c \times f \times 2^{*}$$

2. Tissue sample:

$$VE\ (\mu g/mL) = \frac{OD_{sample}-OD_{control}}{OD_{standard}-OD_{blank}} \times c \times f \times 2^{*} \div \frac{m}{V}$$

Note:

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.

▲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	0.09-40 μg/mL	Average intra-assay CV (%)	4.4
Sensitivity	0.09 μg/mL	Average inter-assay CV (%)	4.9
Average recovery rate (%)	98		

▲Example analysis

Take 0.3 mL of human serum, carry the assay according to the operation table.

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:

VE content(μg/mL)=(0.128-0.066)÷(0.157-0.066)×10×2=13.72 (μg/mL)

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . 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℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . 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℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ to remove blood cells. 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 10000 g at 4℃ . 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℃ for a month.

Note:

1. Homogenized medium: Reagent 4.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 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.)

▲ Notes 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.