# PRODUCT INFORMATION & MANUAL

# Vitamin E Assay Kit (Colorimetric)

## *NBP3-25910*

For research use only.
Not for diagnostic or therapeutic procedures.

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# Vitamin E Assay Kit (Colorimetric)

Catalog No: NBP3-25910

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Sensitivity: 0.95 μg/mL

Detection range: 0.95-40 μg/mL

Average intra-assay CV (%): 3.9

Average inter-assay CV (%): 6.3

Average recovery rate (%): 97

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- 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 α-tocopherol is the most biologically active form of vitamin E.

## ▲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. 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℃, 12 months, shading light |
| Reagent 2 | Ferrum Reagent | Powder × 1 vial | 2-8℃, 12 months, shading light |
| Reagent 3 | Stop Solution | 1.5 mL × 2 vials | 2-8℃, 12 months |
| Reagent 4 | Homogenized Medium | 50 mL × 2 vials | 2-8℃, 12 months |
| Reagent 5 | 1 mg/mL VE Standard | 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.

## ▲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

## ▲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℃ 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℃ 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

## ▲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.

## ▲Detailed operation 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. Reference is as follows:

| Number | Standard concentrations (μg/mL) | 100 μg/mL standard application solution (μL) | Absolute ethanol (μL) |
|---|---|---|---|
| A | 0 | 0 | 500 |
| B | 5 | 25 | 475 |
| C | 10 | 50 | 450 |
| D | 15 | 75 | 425 |
| E | 20 | 100 | 400 |
| F | 25 | 125 | 375 |
| G | 30 | 150 | 350 |
| H | 40 | 200 | 300 |

## 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 μL of n-heptane VE extraction solution to corresponding EP tube.

2) Add 25 μL of reagent 1 application solution and 15 μL 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 μL of reagent 3 and mix fully 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.

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

## ▲Summary 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. | | |
| N-heptane(mL) | 0.5 | 0.5 |
| Mix fully, 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 . | | | |
| N-heptane(mL) | 0.5 | 0.5 | 0.5 |
| Mix fully, 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 (μL) | 200 | 200 | 200 |
| Reagent 1 application solution (μL) | 25 | 25 | 25 |
| Reagent 2 application solution (μL) | 15 | 15 | 15 |
| Mix fully and record time immediately, stand for 5 min accurately at room temperature. | | | |
| Reagent 3 (μL) | 15 | 15 | 15 |
| Mix fully. | | | |
| Absolute ethanol (μL) | 250 | 250 | 250 |
| Mix fully and stand at room temperature for 2 min. Take 200 μL of supernatant to microplate and measure the OD value. | | | |

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:

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

2. Tissue samples:

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

**Note:**

y: $OD_{Standard} - OD_{Blank}$ ($OD_{Blank}$ 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.

$\Delta A_{533}$: $OD_{Sample} - OD_{Blank}$ (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 (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.

# Appendix I Data

## ▲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 = 0.0094 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:

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

# 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 (heparin is recommended), 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 ℃. 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 at4 ℃ .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.)

# Appendix III References


1. Burton G W, Traber M G. Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annual Review of Nutrition, 1990, 10(1): 357-382.
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3. Kimmick G G, Bell R A, Bostick R M. Vitamin E and breast cancer: a review. Nutrition & Cancer, 1997, 27(2): 109-117.
4. Rimbach G, Moehring J, Huebbe P, Lodge J K. Gene-regulatory activity of alpha-tocopherol. Molecules, 2010, 15(3): 1746-1761.
5. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet, 1994, 344(8924): 721-724.