Version 22-04/24

User's Manual

Vitamin E Assay Kit

REF **DEIA-JY2140**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

General Description

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.

Principles of Testing

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.

Reagents And Materials Provided

Reagent 1: Chromogenic Agent, Powder × 1 vial

Reagent 2: Ferrum Reagent, Powder × 1 vial

Reagent 3: Stop Solution, 1.5 mL × 2 vials

Reagent 4: Homogenized Medium, 50 mL × 2 vials

Reagent 5: 1 mg/mL VE Standard, 0.4 mL × 1 vial

Microplate

Plate Sealer

Materials Required But Not Supplied

1. Micropipettor
2. Centrifuge
3. Vortex mixer
4. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
5. Absolute ethanol
6. N-heptane

Storage

2-8°C

Specimen Collection And Preparation

The samples should be prepared as conventional methods.

Serum : Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 ×g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 ×g and 2-8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Tissue homogenates: The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenize them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 ×g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

   *Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 ×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 ×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 ×g at 2-8°C for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000 ×g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

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

Dilution of Samples

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):

Human serum: dilution factor 1

Mouse serum: dilution factor 1

Chicken serum: dilution factor 1

10% Mouse liver tissue homogenate: dilution factor 1

10% Mouse brain tissue homogenate: dilution factor 1

10% Rat kidney tissue homogenate: dilution factor 1

10% Rat lung tissue homogenate: dilution factor 1

10% Rat spleen tissue homogenate: dilution factor 1

10% Rat heart tissue homogenate: dilution factor 1

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

Plate Preparation

	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

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 pg/mL standard application solution

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

Assay Procedure

Assay Note:

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 -heptane in the system.

STEPS

• Step 1: The preparation of standard curve

Dilute 100 pg/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 pg/mL. Reference is as follows:

Standard	Concentration (μg/ml)	100 μg/ml standard application solution	Absolute ethanol
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

• Step 2: Extraction of n-heptane

For serum (plasma) samples

1. 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.
2. Mix fully with a vortex mixer for 20 S.
3. Add 0.5 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 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

For tissue homogenate samples

1. 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.
2. Mix fully with a vortex mixer for 20 S.
3. Add 0.5 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 0.2 mL of n-heptane VE extraction solution (the upper layer solution) for chromogenic reaction.

• Step 3: Chromogenic reaction

1. Take 200 L 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.

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:

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

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.

ΔA_{533}: OD_{Sample} - OD_{Blank} (For serum (plasma) sample, OD_{Blank} is the OD value of 0 ug/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.

A 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:

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

Precision

Intra-Assay: CV ~ 3.9%

Inter-Assay: CV ~ 6.3%

Detection Range

0.95-40 μg/mL

Sensitivity

0.95 μg/mL