Vitamin E(VE) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: NA0515

Size:100T/48S

Components:

Reagent I: 60 mL×1 of anhydrous ethanol, self-prepared, stored at room temperature.

Reagent II: 60 mL×1 of n-heptane, self-prepared, stored at room temperature.

Reagent III: 50 mL×1, storage at 4℃.

Reagent IV: 3 mL×1, stored at 4℃ and protected from light.

Reagent V: powder×1, storage at 4℃ and protected from light.

Preparation of Reagent V stock solution: the powder of Reagent V is dissolved in 2 mL of absolute ethanol to prepare the stock solution, which is stored at 4℃ and protected from light for future use.

Preparation of Reagent V application solution: take the Reagent V stock solution and dilute it with anhydrous ethanol to 20 times before use. It is recommended to configure it for use on the same day.

Reagent VI: 7 mL×1, stored at 4℃.

Standard: Liquid 20 mg×1, stored at -20℃ and protected from light. Add 1 mL of Reagent III into standard before use to prepare 20 mg/mL standard solution.

Product Description

Vitamin E (vitamin E) is a kind of natural fat soluble antioxidant, which can block the peroxidation of unsaturated fatty acids, maintain the integrity and normal function of the membrane of unsaturated fatty acids, and remove superoxide anion free radicals. It has the functions of anti-aging, preventing hemolytic anemia and so on. It has high application value in medicine, cosmetics, health products and food industry.

Fe^{3+} is reduced to Fe^{2+} by VE. Fe^{2+} can react with 1,10-Phenanthroline to produce colored complex, which has a characteristic absorption peak at 510 nm.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, scales, desktop centrifuge, micro glass cuvette/96 well flow-bottom plate, adjustable pipette, mortar/homogenizer, vortex shaker, absolute ethanol, n-heptane, distilled water and EP tube.

Procedure

I. Extraction of vitamin E

1. Tissue samples

Reagent name	
Tissue (g)	0.1

Distilled water (μL)	200
Reagent I (μL)	300
Reagent II (μL)	500
After homogenization, shake for 5 minutes on the vortex mixer (full extraction), centrifuge at 5000 ×g for 5 minutes at 25℃, take 300 μL of the upper n-heptane extract solution and add it to 900 μL of absolute ethanol (the upper extraction: absolute ethanol = 1:3), mix and wait for measurement.	

Note: after centrifugation, do not inhale the liquid phase layer of anhydrous ethanol and water when drawing the upper n-heptane extract.

2. Serum (plasma) sample

Reagent name	
Serum (plasma) (g)	200
Distilled water (μL)	200
Reagent I (μL)	300
Reagent II (μL)	500
After homogenization, shake for 5 minutes on the vortex mixer (full extraction), centrifuge at 5000 ×g for 5 minutes at 25℃, take 300 μL of the upper n-heptane extract solution and add it to 900 μL of absolute ethanol (the upper extraction: absolute ethanol = 1:3), mix and wait for measurement.	

Note: after centrifugation, do not inhale the liquid phase layer of anhydrous ethanol and water when drawing the upper n-heptane extract.

II. Measurement steps

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 510 nm, and adjust zero with absolute ethanol.

2. Dilute 20 mg/mL standard solution with Reagent III to 50, 25, 12.5, 6.25 and 3.125 μg/mL standard solution for standby.

3. Operation table: add the following reagents in turn

Reagent name (μL)	Contrast tube（C）	Test tube（T）	Standard tube（S）	Blank tube（B）
Sample to be tested	100	100	-	-
Standard	-	-	100	-
Reagent III	-	-	-	100
Reagent IV	20	20	20	20
Reagent V	-	20	20	-
Reagent I	20	-	-	20
Mix well, record the time immediately, react at 25℃ for 5 min.				
Reagent VI (μL)	60	60	60	60

Mix well, measure the absorbance value at 510 nm, record as A_C and A_T, A_S and A_B. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each measuring tube shall be provided with a Contrast tube.

III. Calculation of Vitamin E(VE) Content:

1. Drawing of standard curve:

Take the concentration of each standard solution as the x-axis, and the corresponding ΔA_S as the y-axis, draw the standard curve, get the standard equation y=kx+b, and bring ΔA into the equation to get x (μg/mL).

Calculation of vitamin E content:

(1) Calculated by sample mass

VE content (μg/g fresh weight) = $x \times 4 \times V_{ST} \div W = 2x \div W$

(2) Calculated by the volume of serum (plasma)

VE content (μg/mL)=$x \times 4 \times V_{ST} \div V_{S(P)} = 10x$

4: The sample to be tested is 300 μL of n-heptane extract plus 900 μL of anhydrous ethanol, which is equivalent to diluting the extracted sample four times before testing;

V_{ST}: the volume of n-heptane added in the extraction process, 0.5 mL;

W: the sample mass, g;

$V_{S(P)}$: the volume of serum (plasma) added in the extraction process, 0.2 mL.

Note:

1. After centrifugation, when the upper n-heptane extract is absorbed, do not inhale the liquid phase layer of anhydrous ethanol and water in the middle to avoid affecting the test results.
2. If A>0.8, it is recommended to dilute the sample to be tested with Reagent III appropriately and multiply the dilution multiple in the calculation formula.
3. If the reaction system produces precipitation, it is recommended to dilute the sample to be tested with Reagent III appropriately, and multiply the dilution multiple in the calculation formula.
4. The cuvette shall be rinsed with anhydrous ethanol, and distilled water shall not be used to prevent layering from affecting the test data.
5. The determination shall be completed as soon as possible after the completion of color development.

Examples:

1.Take 0.1g walnut, add 200μL distilled water, 300μL Reagent I and 500μL Reagent II and grind thoroughly, shake on vortex mixer for 5 min(full extraction), centrifuge with 5000rpm at 25℃ for 5min, take 300μL upper extract solution and add it to 900μL ethanol absolute(extract solution:ethanol absolute=1:3), follow the determination procedure to operate, with 96-well flat-bottom plates to calculate: ΔA =A(T)-A(B)=0.336-0.046=0.29, standard curve: y=0.0042x+0.1849, calculate x=25.02, according with mass of sample to calculate: VE content (μg/g mass)=2×x÷W==2×25.02÷0.1=500.4 μg/g mass.

Related Products:

NA0335/NA0334 Vitamin B1 (VB1) Content Assay Kit

NA0720/NA0478 Vitamin B6(VB6) Content Assay Kit