CheKine™ Micro Vitamin E (VE) Content Assay Kit

Cat #: KTB2400 Size: 48 T/96 T

	Micro Vitamin E (VE) Content Assay Kit		
REF	**Cat #:** KTB2400	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Vitamin E (VE) is a fat-soluble vitamin whose hydrolysis product is tocopherol, one of the primary antioxidants in biological systems. It serves to prevent unsaturated fatty acids from oxidative damage, thereby maintaining the integrity and normal functionality of cell membranes composed of such acids. It possesses the ability to retard aging processes and is effective in preventing hemolytic anemia. Vitamin E holds considerable application value in the pharmaceutical, cosmetic, health supplement, and food industries.VE reduces Fe^{3+} ions to Fe^{2+}, which subsequently forms a colored complex with 1,10-phenanthroline. This complex exhibits a characteristic absorption peak at a wavelength of 530 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1 mL	2 mL	4℃, protected from light
Reagent Ⅱ	1 mL	2 mL	4℃
Reagent Ⅲ	1 mL	2 mL	4℃
Reagent Ⅳ	3 mL	6 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 530 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, centrifuge, vortex shaker
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissue samples: Weigh 0.1 g tissue sample, add 1 mL Extraction Buffer, after homogenization, the mixture was diluted to a final volume of 1 mL using Extraction Buffer. The solution was vortexed for 5 min on a vortex mixer, then centrifuged at 5,000 g for 10 min at 25℃. The supernatant was collected and reserved for further analysis.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 3 min (power 300 W, ultrasonic 3 s, interval 7 s, total time is 3 min). The solution was vortexed for 5 min on a vortex mixer, then centrifuged at 5,000 g for 10 min at 25℃. The supernatant was collected and reserved for further analysis.
3. Serum (plasma) and other liquid samples: Take 0.1 mL and add it to 0.9 mL of Extraction Buffer, the solution was vortexed for 5 min on a vortex mixer, then centrifuged at 5,000 g for 10 min at 25℃. The supernatant was collected and reserved for further analysis.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 530 nm, visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

Reagent	Control Well (μL)	Test Well (μL)
Sample Supernatant	100	100
Reagent Ⅰ	20	20
Reagent Ⅱ	0	20
Reagent Ⅲ	20	0
Mix thoroughly and incubate at 25℃ for 5 min.		
Reagent Ⅳ	60	60
Mix well and measure the absorbance at 530 nm. Record these values as $A_{Control}$ and A_{Test}, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$.		

Note: Each test well needs to be equipped with a control well, before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If precipitates form in the reaction system, the sample should be appropriately diluted with Extraction Buffer. In this case, the dilution factor must be multiplied into the calculation formula.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. Microglass cuvette calculation formula as below

Regression equation determined under standard conditions: y=0.22x+0.0065, R^2=0.9978, x represents the standard (μg/mL), y denotes ΔA.

(1) Calculated by protein concentration

VE (μg/mg prot)=(ΔA-0.0065)÷0.22×V_{Total}÷(V_{Sample}×Cpr)**=9.09×(ΔA-0.0065)÷Cpr**

(2) Calculated by sample fresh weight

VE (μg/g fresh weight)=(ΔA-0.0065)÷0.22×V_{Total}÷(V_{Sample}×W÷$V_{Sample\ Total}$)**=9.09×(ΔA-0.0065)÷W**

(3) Calculated by cell number

VE (μg/10^4 cell)=(ΔA-0.0065)÷0.22×V_{Total}÷(V_{Sample}×N÷$V_{Sample\ Total}$)**=9.09×(ΔA-0.0065)÷N**

(4) Calculated by sample volume

VE (μg/mL)=(ΔA-0.0065)÷0.22×V_{Total}÷V_{Sample}×10**=90.9×(ΔA-0.0065)**

Where: V_{Total}: the total volume of the reaction system, 0.2 mL; V_{Sample}: the volume of the sample in the reaction system, 0.1 mL; $V_{Sample\ Total}$: The volume of Extraction Buffer added, 1 mL; Cpr: protein concentration, mg/mL; W: sample weight, g; N: Total number of cells, 10^4.

B. Microquartz cuvette calculation formula

Regression equation determined under standard conditions: y=0.11x+0.0065, R^2=0.9978, x represents the standard (μg/mL), y denotes ΔA.

(1) Calculated by protein concentration

VE (μg/mg prot)=(ΔA-0.0065)÷0.22×V_{Total}÷(V_{Sample}×Cpr)**=18.18×(ΔA-0.0065)÷Cpr**

(2) Calculated by sample fresh weight

VE (μg/g fresh weight)=(ΔA-0.0065)÷0.22×V_{Total}÷(V_{Sample}×W÷$V_{Sample\ Total}$)**=18.18×(ΔA-0.0065)÷W**

(3) Calculated by cell number

VE (μg/10^4 cell)=(ΔA-0.0065)÷0.22×V_{Total}÷(V_{Sample}×N÷$V_{Sample\ Total}$)**=18.18×(ΔA-0.0065)÷N**

(4) Calculated by sample volume

VE (μg/mL)=(ΔA-0.0065)÷0.22×V_{Total}÷V_{Sample}×10**=181.8×(ΔA-0.0065)**

Where: V_{Total}: the total volume of the reaction system, 0.2 mL; V_{Sample}: the volume of the sample in the reaction system, 0.1 mL; $V_{Sample\ Total}$: The volume of Extraction Buffer added, 1 mL; Cpr: protein concentration, mg/mL; W: sample weight, g; N: Total number of cells, 10^4.

Precautions

1. Centrifugation complete, when aspirating the upper layer of n-heptane extract, be careful not to draw in the middle liquid phase containing anhydrous ethanol and water to avoid affecting the experimental results.
2. The cuvettes must be rinsed with anhydrous ethanol; do not use deionized water as this may cause layering that could impact the test data.
3. It is crucial to complete the measurement as soon as possible after the color development has ended.

Typical Data

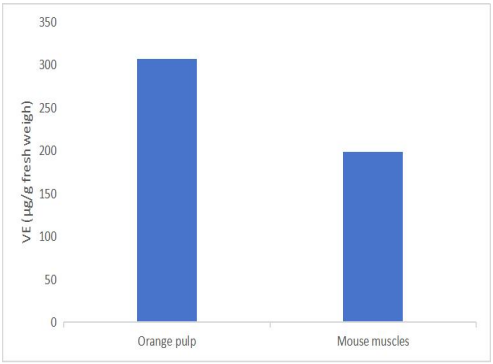

Figure 1. Determination VE content in Orange pulp and Mouse muscles by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.