Vitamin E Microplate Assay Kit

User Manual

Catalog # CAK1169

(Version 1.3A)

Detection and Quantification of Vitamin E Content in Serum, Plasma, Tissue extracts, Other biological fluids Samples.

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

I. INTRODUCTION

Vitamin E is a group of eight compounds that include four tocopherols and four tocotrienols. Alpha-tocopherol (α-tocopherol), the most biologically active form of vitamin E, is the second-most common form of vitamin E in the diet. This variant can be found most abundantly in wheat germ oil, sunflower oil, and safflower oil. As fat-soluble antioxidants, tocopherols interrupt the propagation of reactive oxygen species that spread through biological membranes or through fat when its lipid content undergoes oxidation by reacting with lipid radicals.

Vitamin E Microplate Assay Kit is a sensitive assay for determining Vitamin E content in various samples. Vitamin E reduces Fe^{3+} to Fe^{2+}, and Fe^{2+} produces a colored complex with phenanthroline. The color intensity at 530 nm is directly proportional to Vitamin E concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	Powder x 1	4 °C
Assay Buffer II	10 ml x 1	4 °C
Extract	30 ml x 4	4 °C
Reaction Buffer	5 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	10 µl x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Assay Buffer I: add 30 ml ethanol to dissolve before use.

Substrate: add 5 ml ethanol to dissolve before use.

Dye Reagent: add 5 ml ethanol to dissolve before use.

Standard: add 990 µl ethanol to dissolve, then add 100 µl Standard into 900 µl ethanol, the concentration will be 2 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 530 nm
2. Ethanol
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For serum, plasma or Other biological fluids samples

Add 50 µl sample into centrifuge tube, then add 200 µl Assay Buffer I, shake and mix by vortexing for 2 minutes; then add 1 ml Extract, shake and mix by vortexing for 2 minutes, centrifuged at 8000g for 10 minutes. Absorb the supernatant into a new centrifuge tube.

2. For tissue samples

Weigh out 0.05 g tissue, homogenize with 200 µl Assay Buffer I and 200 µl Assay Buffer II, shake and mix by vortexing for 20 minutes, centrifuged at 8000g 4 °C for 10 minutes, absorb the supernatant into a new centrifuge tube; then add 1 ml Extract, shake and mix by vortexing for 2 minutes, centrifuged at 8000g for 10 minutes. Absorb the supernatant into a new centrifuge tube.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	50 µl	50 µl	50 µl
Substrate	50 µl	50 µl	50 µl
Dye Reagent	50 µl	50 µl	50 µl
Sample	50 µl	--	--
Standard	--	50 µl	--
Ethanol	--	--	50 µl
Mix, record absorbance measured at 530 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Vitamin E } (\mu\text{mol/mg}) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) \times n$$

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein} \times n$$

2. According to the weight of sample

$$\text{Vitamin E } (\mu\text{mol/g}) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W) \times n$$

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W \times n$$

3. According to the volume of sample

$$\text{Vitamin E } (\mu\text{mol/L}) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times n$$

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) \times n$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$C_{Standard}$: the standard concentration, 2 mmol/L = 2 μmol/ml;

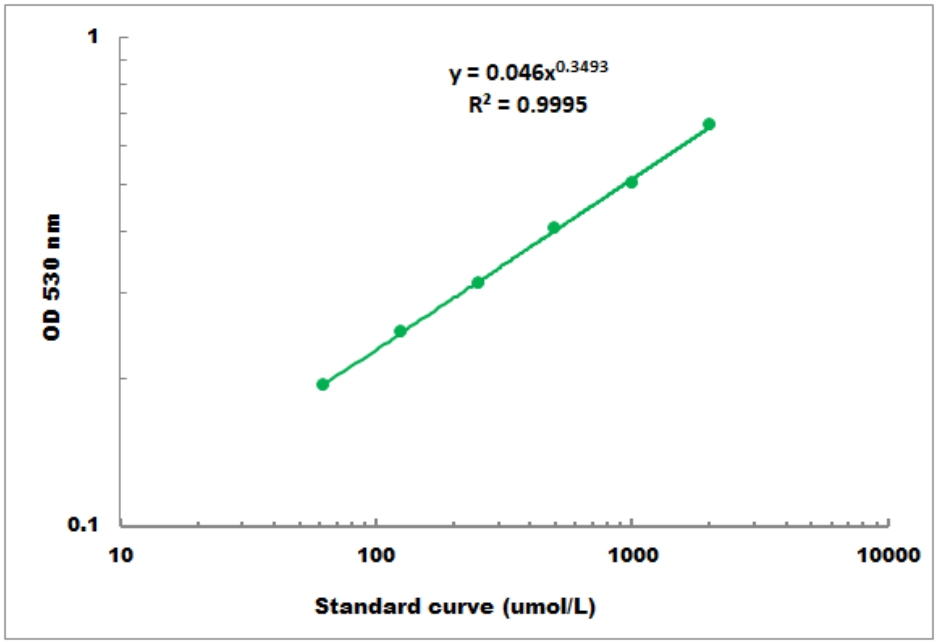
$V_{Standard}$: the volume of the standard, 0.05 ml;

V_{Sample}: the volume of sample, 0.05 ml;

n: dilution ratio.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 50 µmol/L - 2000 µmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES