

Vitamin E Microplate Assay Kit

Cat #: orb707345 (manual)

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.

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.

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	
Technical Manual	1 Manual	

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.

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

SAMPLE PREPARATION

1. For serum, plasma or Other biological fluids samples

Add 50 μ l sample into centrifuge tube, then add 200 μ l 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 μ l Assay Buffer I and 200 μ l 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.

ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	50 μ l	50 μ l	50 μ l
Substrate	50 μ l	50 μ l	50 μ l
Dye Reagent	50 μ l	50 μ l	50 μ l
Sample	50 μ l	--	--
Standard	--	50 μ l	--
Ethanol	--	--	50 μ l

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.

CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned}\text{Vitamin E } (\mu\text{mol/mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad C_{\text{Protein}}) \times n \\ &= 2 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}} \times n\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{Vitamin E } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (V_{\text{Sample}} \times W) \times n \\ &= 2 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \times n\end{aligned}$$

3. According to the volume of sample

$$\begin{aligned}\text{Vitamin E } (\mu\text{mol/L}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / V_{\text{Sample}} \times n \\ &= 2 \times (OD_{\text{Sample}} - OD_{\text{Control}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \times n\end{aligned}$$

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

W: the weight of sample, g;

C_{Standard} : the standard concentration, 2 mmol/L = 2 $\mu\text{mol/ml}$;

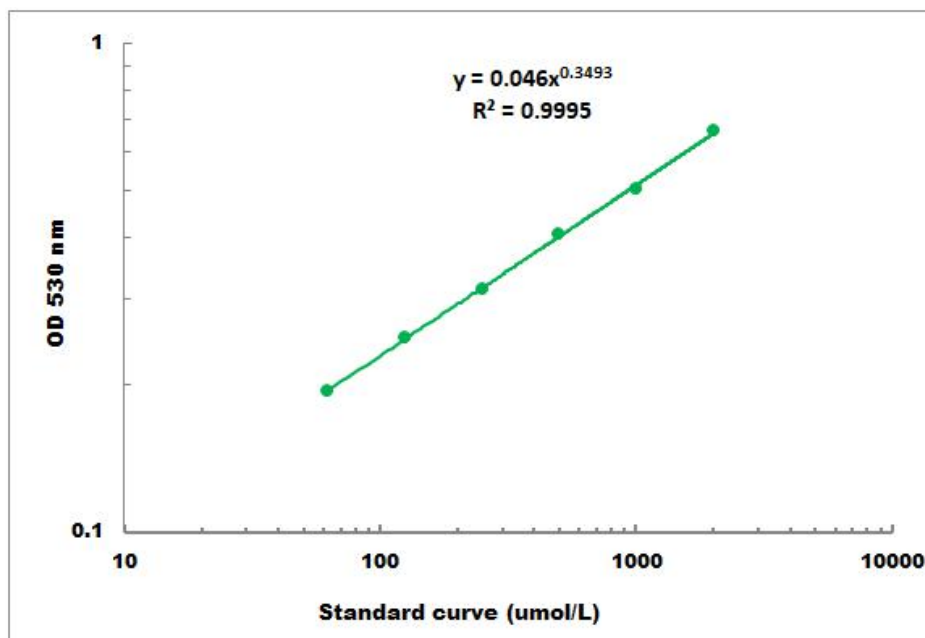
V_{Standard} : the volume of the standard, 0.05 ml;

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

n: dilution ratio.

TYPICAL DATA

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



Detection Range: 50 $\mu\text{mol/L}$ - 2000 $\mu\text{mol/L}$