Vitamin E

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1169

(Version 1.4A)

Detection and Quantification of Vitamin EContentinSerum, Plasma, Tissue extracts, Other biological fluidsSamples.

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 EColorimetric Microplate Assay Kit is a sensitive assay for determining Vitamin Econtent 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	Powderx 1	4 °C
Assay Buffer II	10 mlx 1	4 °C
Extract	30 ml x 4	4 °C
Reaction Buffer	5 mlx 1	4 °C
Substrate	Powderx 1	4 °C, keep in dark
Dye Reagent	Powderx 1	4 °C, keep in dark
Standard	10 µlx 1	4 °C, keep in dark
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Assay Buffer I: Add 30 mlethanol to dissolve before use.Store at 4 °C for 4-5 daysafter reconstitution.

Substrate: Add 5 mlethanol to dissolve before use.Store at 4 °C for 1-2 monthsafter reconstitution.

Dye Reagent: Add 5 mlethanol to dissolve before use.Store at 4 °C for 6 monthsafter reconstitution.

Standard: Briefly centrifuge prior to opening.Dissolvein990µlethanolto generate 20 mmol/L of standardstock solution, store at 4 °C for 1 monthafter reconstitution.Then dilute to2 mmol/L standard top solutionby adding 100 µl stock solution into 900µl ethanol.Perform 2-fold serial dilutions of the top standardsolution using ethanolto make the standard curve.The concentration of standard curve could be2/1/0.5/0.25/0.125/0.0625/0.0312mmol/L.

V. SAMPLE PREPARATION

1.For serum, plasma or Other biological fluidssamples

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

2.For tissue samples

Weigh out 0.05g tissue, homogenize with 200 µlAssay Buffer I and 200 µlAssay Buffer II, shake and mix by vertexing 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 vertexing for 2 minutes, centrifuged at 8000g for 10minutes. Absorb the supernatant into a new centrifuge tube.

VI. 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.2According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

$V_{Standard}$: the volume of standard in assay procedure, μl

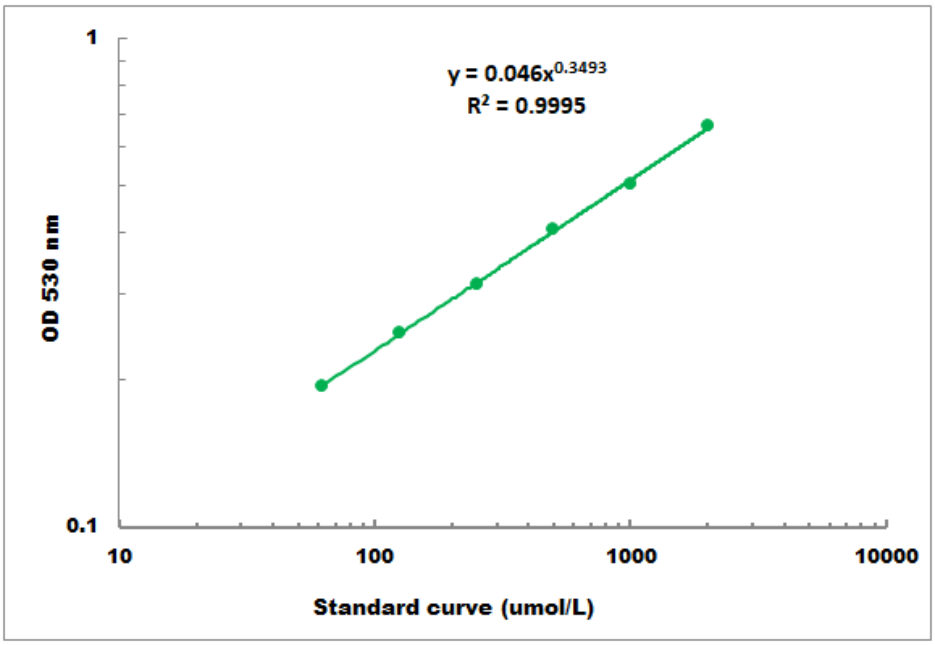
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

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



Detection Range: 50μmol/L-2000μmol/L