Vitamin E (Alpha-Tocopherol) Assay Kit

Catalog No: abx298985

Size: 96T

Detection Range: 0.95 µg/ml – 40 µg/ml

Sensitivity: 0.95 µg/ml

Storage: Store all components at 4°C in the dark.

Application: The quantitative detection of Vitamin E (Alpha-Tocopherol) in serum, plasma and tissue homogenates.

Background: Vitamin E is a group of eight lipid soluble antioxidants critical in maintaining the structure of cell membranes and influencing gene expression. Alpha-Tocopherol is the most biologically active form of Vitamin E, playing a role in metabolism in the glutathione peroxidase pathway.

Principle of the Assay: Fe^{3+} can be reduced to Fe^{2+} by Vitamin E (Alpha-Tocopherol) in the presence of Ferroin. Fe^{2+} reacts with phenanthroline to form a pink compound with an absorbance maximum at 533 nm. The Optical Density (OD) is measured spectrophotometrically at 533 nm in a microplate reader, from which the concentration of Vitamin E (Alpha-Tocopherol) can be calculated.

Kit Components

- 96-Well Microplate: 12 x 8
- Standard (1 mg/ml): 0.4 ml
- Homogenization medium: 2 × 50 ml
- Detection Reagent: 1 vial
- Iron Reagent: 1 vial
- Stop Solution: 2 × 1.5 ml
- Plate Sealer: 2

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Double distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 533 nm)
- Microplate Shaker
- Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
- Centrifuge
- Timer

Reagents Required But Not Provided

- Absolute ethanol
- N-heptane

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Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.
- **Tissue Homogenates**: The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh tissue before homogenization. Finely mince tissues and for each 1 g of tissue, homogenize with a tissue homogenizer on ice in 9 ml of Homogenization medium. Centrifuge the homogenates at 10000 × g for 5 mins at 4°C and take the supernatant on ice for detection.

Notes:

- Samples must be diluted so that the expected concentration falls within the kit's range. Dilute samples with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
- We recommend testing the following samples undiluted: Human serum, Chicken serum, Mouse serum, 10% Mouse liver homogenate, 10% Mouse brain homogenate, 10% Rat kidney homogenate, 10% Rat lung tissue homogenate, 10% Rat spleen homogenate, 10% Rat heart homogenate.
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- **Samples should not contain reducing agents such as DDT, 2-mercaptoethanol, or chelating agents such as EDTA or HEDP.**
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

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B. Reagent Preparation

Detection Reagent Solution: Dissolve 1 vial of Detection Reagent with 13 ml of Absolute ethanol and mix fully until fully dissolved. The solution may be stored at 4°C for up to 7 days. *Note that the powder may be difficult to dissolve, prepare 3-4 hours before use and ensure the powder is fully dissolved.*

Iron Reagent Solution (10X): Dissolve 1 vial of Iron Reagent with 25 ml of Absolute ethanol and mix fully until fully dissolved. The solution may be stored at 4°C in the dark for up to 7 days.

Iron Reagent Solution (1X): Dilute the 10X Iron Reagent Solution 10-fold with Absolute ethanol and mix fully. Prepare fresh before use.

Standard Solution (100 µg/ml): Dilute the 1 mg/ml Standard Solution 10-fold with Absolute ethanol and mix fully. Prepare fresh before use.

Standard Curve: Dilute the 100 µg/ml Standard Solution with Absolute ethanol according to the following table:

Volume of 100 µg/ml Standard Solution (µl)	Volume of Absolute ethanol (µl)	Standard concentration (µg/ml)
0	500	0
25	475	5
50	450	10
75	425	15
100	400	20
125	375	25
150	350	30
200	300	40

C. Assay Protocol

Prepare all standards, samples and reagents as directed above. Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate, and to plot a standard curve for each test. Tubes must be cleaned with boiling water, wash with running water, and then wash with double distilled water. When adding reagents or sample, pipette slowly and vertically, ensuring not to touch the sides or bottom of the tubes.

1. Set standard and test sample wells on the microplate respectively, and record their positions.
2. Label 8 tubes according to the standard concentrations (0 µg/ml, 25 µg/ml etc.)
3. **Standard tubes:** Add 0.15 ml of double distilled water and 0.3 ml of each standard to the respective standard tube.
4. **Sample tubes:** Add 0.15 ml of each sample and 0.3 ml of Absolute ethanol to the respective sample tube.
5. **Blank tube:** *Only necessary for tissue homogenate samples.* Add 0.15 ml of Homogenization medium and 0.3 ml of Absolute ethanol to the blank tube.
6. Seal all tubes, and mix with a vortex for 20 seconds.
7. Add 0.5 ml of N-heptane to all tubes, seal the tubes, and mix with a vortex mixer for 1 minute accurately.
8. Centrifuge all tubes at 3100 × g for 10 minutes at room temperature. Take 200 µl of the top layer supernatant to a new tube for detection. *Do not take any of the bottom layer, as this can influence results. The new tube must be dry before use.*
9. Add 25 µl of Detection Reagent Solution, and 15 µl of Iron Reagent Solution (1X) to each tube.
10. Mix fully with a vortex, seal the tubes, and begin the timer. Leave to stand at room temperature for 5 minutes exactly.
11. Add 15 µl of Stop Solution, seal the tubes and mix with a vortex for 10 seconds.
12. Add 250 µl of Absolute ethanol, seal the tubes, and vortex until mixed fully.
13. Stand at room temperature for 2 minutes. Take 200 µl of each solution to their respective well on the microplate. *Pipette slowly and vertically, ensuring not to touch the sides or bottom of the well.*
14. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 533 nm immediately.

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D. Calculations

The standard curve can be plotted as the relative OD of each reference standard solution (X), against the respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor, to obtain the concentration before dilution.

1. **Serum and Plasma**

$$\text{Vitamin E } (\mu g/ml) = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - b}{a} \times f \times 2$$

2. **Tissue homogenates**

$$\text{Vitamin E } (\mu g/g) = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - b}{a} \times f \times 2 \times \frac{V}{m}$$

where:

a	The gradient of the standard curve ($y = ax + b$)
b	The intercept of the standard curve ($y = ax + b$)
f	The dilution factor prior to assay
$\text{OD}_{\text{Sample}}$	The sample absorbance
OD_{Blank}	The blank absorbance. For serum/plasma, this is the value of the 0 $\mu g/ml$ standard. For tissue homogenates, this is the value of the blank tube.
m	The weight of the sample (g)
V	The volume of the homogenization medium (ml)
2	The volume of standard (0.3 ml) is double the volume of sample (0.15 ml)

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled. Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- Please note that this kit is optimised for detection of native samples, rather than synthetic chemicals. We are unable to guarantee detection of synthetic compounds, as they may have different structures to the native compound or contaminants present.
- All tubes should be cleaned before use according to the instructions. Tubes should be dry prior to assay.