Human Vitamin E ELISA

Cat No: K12-S013

Principle:

This ELISA kit uses Sandwich-ELISA as the method. The Microtiter plate provided in this kit has been pre-coated with an antibody specific to Vitamin E. Standards or samples are added to the appropriate Microtiter plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for Vitamin E is added to each Microtiter plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain Vitamin E and HRP conjugated Vitamin E antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Estrogen. You can calculate the concentration of Vitamin E in the samples by comparing the OD of the samples to the standard curve.

Intended Use:

This kit is used to assay the level of Human Vitamin E in human serum, blood, plasma, and other test specimens.

Materials Provided:

1. Microtiter Coated Plate (96 wells) – 1 no
2. Standard, 1080 ng/ml – 0.5 ml
3. HRP Conjugate - 6 ml
4. Wash Buffer (30X) – 20 ml
5. Standard Diluent – 1.5 ml
6. Sample diluent – 6ml
7. Substrate A – 6 ml
8. Substrate B – 6 ml
9. Stop Solution – 6 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 µl to 1000 µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi-log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

Storage Information:

1. All reagents should be stored at 2 ºC to 8 ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25 ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all samples in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. The kit cannot test samples which contain NaN3, because NaN3 inhibits HRP activity.
2. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
3. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
4. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 20-min at the 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
6. **Cell Culture supernatant-** Collect sample in a sterile container. Centrifuge for 20 mins at 2000-3000 rpm. Remove the supernatant. Dilute cell suspension with PBS（PH7.2-7.4, if cell concentration is greater than 1 million/ml. Repeated freeze-thaw cycles, damage the cells and release intracellular components.
7. **Tissue samples-** After cutting the sample, check the weight and add PBS (PH7.2-7.4). Rapidly freeze with liquid nitrogen. Maintain samples at 2-8℃ after melting. Add PBS (PH7.4). Homogenize by hand or grinder. Centrifuge for 20 mins at 2000-3000 rpm. Remove the supernatant.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.
2. To make 1X Wash Solution, add 10 ml of 30X Wash Buffer in 290 ml of DI water.

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. If the concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of Samples containing sodium azide (NaN3), as it could destroy the HRP activity of the conjugate resulting in under-estimation of the antibodies.
4. All Standards and Samples should be assayed at least in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromising the sensitivity of the assay.
7. The plates should be read within 15 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples should be run in duplicates or triplicates. A standard curve is required for each assay.
2. Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and standard diluent.

720 ng/ml	Standard No.5	*120µl Original Standard (1080 ng/ml) + 60µl Standard diluent*
480 ng/ml	Standard No.4	*100µl Standard No.5 + 50µl Standard diluent*
240 ng/ml	Standard No.3	50µl Standard No.4 + 50µl Standard diluent
120 ng/ml	Standard No.2	50µl Standard No.3 + 50µl Standard diluent
60 ng/ml	Standard No.1	50µl Standard No.2 + 50µl Standard diluent

3. Remove the number of strips required for the assay.
4. Pipette out 50 µl of **Standards** and **40µl sample diluent and 10 µl of Samples** into the respective wells as mentioned in the work list.
5. Cover the plate and incubate for 30 minute at 37 °C in the incubator.
6. Aspirate and wash plate 4 times with **1X Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Pipette out 50 µl of **HRP Conjugate** into each sample and standards well. Do not pipette into the Blank well.
8. Cover the plate and incubate for 30 minute at 37 °C in the incubator.
9. Aspirate and wash plate 4 times with **1X Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
10. Then add **Substrate A** 50 µl, then **Substrate B** 50 µl to each well including Blank well. Gently mixed, incubate for 15 min at 37 ℃ in dark.
11. Pipette out 50 µl of **Stop Solution**. Wells should turn from blue to yellow in colour.
12. Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution blanking on the zero standards.

Calculation of Results:

Calculate the mean optical density of each standard duplicate. Draw a standard curve on semi-log paper with the mean optical densities on the Y-axis and the standards concentration on the X-axis. If ELISA software is being used, a 4-parameter curve is recommended. Calculate the mean optical density of each unknown duplicate. Read the values of the unknowns directly off the standard curve.

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be **0.93 ng/ml**.

Assay range: 15.63ng/ml –1000 ng/ml

Precision:

Intra-assay Precision: 3 samples with low, middle and high level Human Vitamin E were tested 20 times on one plate, respectively.

Inter-assay Precision: 3 samples with low, middle and high level Human Vitamin E were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/mean X 100
Intra-Assay: CV<10%
Inter-Assay: CV<12%