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Product Information

25(OH) Vitamin D ELISA kit

Catalog Number: EA100877
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The 25-hydroxy (25-OH) Vitamin D ELISA is intended for the quantitative determination of total 25-OH Vitamin D in human serum and plasma.

Background

Vitamin D is a steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. Vitamin D has two isomers: Vitamin D2 and Vitamin D3. Vitamin D2 is obtained from dairy products whereas Vitamin D3 is produced in the skin after exposure to ultraviolet light. In the liver, Vitamin D is hydroxylated at its carbon 25 to form 25-OH Vitamin D. This metabolite is the predominant circulating form of Vitamin D and is considered to be an accurate indicator of the general Vitamin D status of an individual. Vitamin D deficiency has been linked to many diseases including osteoporosis, rickets, osteomalacia, cancers, and cardiovascular diseases. Both dietary supplements of Vitamin D that are currently available in the market (Vitamin D2 and Vitamin D3) are converted to 25-OH Vitamin D in the liver. The sum of the concentrations of 25-OH Vitamin D2 and 25-OH Vitamin D3, in serum or plasma, is referred to as “Total 25-OH Vitamin D”. Accurate monitoring of total 25-OH Vitamin D level is critical in clinical settings. Vitamin D deficient patients who are prescribed a daily Vitamin D supplement should regularly monitor their serum or plasma Vitamin D levels in order to reach an optimal level and prevent their 25-OH Vitamin D concentrations from reaching excessive levels that are considered toxic.[1-5]

Principle of the Test

The kit is a solid phase enzyme-linked immunoassay (ELISA), based on the principal of competitive binding. Anti- Vitamin D antibody coated wells are incubated with Vitamin D standards, controls, samples, and Vitamin D-Biotin conjugate at room temperature for 90 minutes. During the incubation, a fixed amount of biotin-labeled vitamin D competes with the endogenous Vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti Vitamin D antibody. Following a wash step, bound Vitamin D-Biotin is detected with Streptavidin-HRP (SA-HRP). SA-HRP conjugate immunologically bound to the well progressively decreases as the concentration of Vitamin D in the specimen increases. Unbound SA-HRP conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 30 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The color intensity will be inversely proportional to the amount of 25-OH Vitamin D in the sample. The assay measures both the 25-OH Vitamin D2 and D3. The total assay procedure run time is 2.5 hours.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell plate coated with anti-Vitamin D	12x8x1
2. Vitamin D Standard Set: 7 vials (ready to use)	0.5 ml
3. Vitamin D Control Set: 2 vials (ready to use)	0.5 ml
4. Biotinylated 25(OH)D Reagent: 1 Vial (51X)	0.55 ml
5. Assay Diluent, 1 bottle	24 ml
6. Streptavidin-HRP, 1 bottle (ready to use)	23 mL
7. Stop Solution, 1 bottle (ready to use)	12 mL
8. TMB Substrate, 2 bottles (ready to use)	2 x 12 ml
10. Wash Concentrate 20X, 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Precision pipettes
2. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Flat-head Vortex mixer
5. Plate shaker
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Plasma should be treated with EDTA.
3. Plasma samples may be stored at 2-8°C for up to 8 hours, and should be frozen at -20°C or lower for up to 4 months.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Do not use grossly hemolyzed or lipemic specimens.

Reagent Preparation

Before running the test, prepare the following:

1. **Standards and Reagents:**
   Standards are serum-based solutions and stable when stored at -2-8°C, protected from light, until the expiration date on the label. Equilibrate the needed volume of standards and reagents to room temperature before use.
2. **51X Biotin conjugate:** Immediately before use, prepare 1X working solution at 1:51 with assay diluent (e.g. Add 0.1ml of the 51X Vitamin D-Biotin conjugate concentrate to 5ml of assay diluent). **Remaining Assay Diluent must be stored at 2-8°C in dark and tightly capped.**
3. **Prepare 1X Wash Buffer:** Add the Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Dispense 10 µl of 25-OH Vitamin D Standards, controls and samples into each well, as required.
2. Dispense 200 µl working solution of biotinylated 25 (OH) D reagents into each well.
3. Carefully mix the contents in the wells for 20 seconds using a plate shaker at 200-400 RPM (or equivalent motion). Remove from shaker and cover the plate with the adhesive plate seal making sure there is a complete seal over each well.
4. **INCUBATION #1** – Incubate sealed plate for 90 minutes at room temperature.
5. Briskly shake out the contents of the wells into a waste reservoir.
6. **WASH # 1** - Dispense 300 µl of 1X Wash Buffer into each well, and then briskly shake out the 1X Wash Buffer into a waste reservoir. Strike the wells sharply on absorbent paper to remove residual droplets. Repeat 2 more times for a total of 3 washes.
7. Dispense 200 µl of enzyme conjugate (Streptavidin-HRP) into each well.
8. **INCUBATION #2** - Incubate for 30 minutes, at room temperature.
9. Briskly shake out the contents of the wells into a waste reservoir.
10. **WASH # 2** - Dispense 300 µl of 1X Wash Buffer into each well, and then briskly shake out the 1X Wash Buffer into a waste reservoir. Strike the wells sharply on absorbent paper to remove residual droplets. Repeat 2 more times for a total of 3 washes.
11. Using a multi-channel pipette, dispense 200 µl of TMB Substrate into each well.
12. **INCUBATION #3** - Incubate for 30 minutes at room temperature, preferably in the dark.
13. **STOP** - Dispense 50 µl of Stop Solution into each well to stop the enzymatic reaction. Carefully mix plate contents for 20 - 30 seconds.
14. Read absorbance on ELISA Reader at 450 nm within 10 minutes of adding the Stop Solution.

Calculation of Results

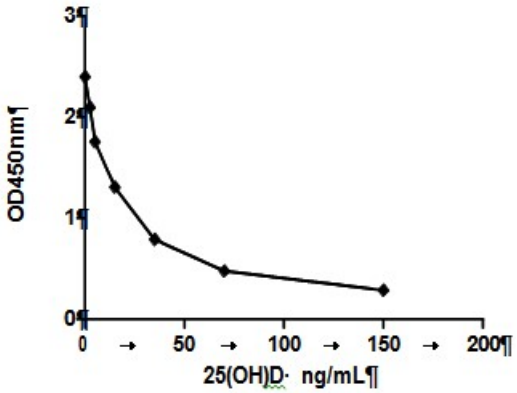
The standard curve is constructed as follows:

1. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml with absorbance value on the Y-axis and concentration on the X-axis.
2. Calculate the average absorbance values for each set of reference standards, controls and patient samples.
3. Using the mean absorbance value for each sample determine the corresponding concentration of 25(OH) Vitamin D in ng/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further converted by the appropriate dilution factor.

Example of a Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standards	Conc (ng/mL)	OD450 (nm)
Standard 1	0	2.40
Standard 2	2.5	2.10
Standard 3	5	1.76
Standard 4	15	1.31
Standard 5	35	0.79
Standard 6	70	0.48
Standard 7	150	0.29



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