


REFERENCES:

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25(OH) Vitamin D ELISA

Catalog No.: VD220B (96 Tests)

INTENDED USE

The Calbiotech, Inc. 25-hydroxy (25-OH) Vitamin D ELISA is intended for the quantitative determination of total 25-OH Vitamin D in human serum and plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

25-hydroxyvitamin D is the major circulating form of vitamin D. Research includes vitamin D metabolism, regulation of calcium homeostasis, VDR (vitamin D receptor) signaling pathways, and epidemiologic studies on vitamin D status in populations.

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.

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
9.	Wash Concentrate 20X, 1 bottle	25 mL

MATERIALS 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

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

This assay is designed for use in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

Serum, heparinized plasma or EDTA plasma samples can be used for the assay.

- For serum, collect whole blood by venipuncture and allow clotting.
- For plasma, mix the sample by gentle inversion prior to centrifugation.

Centrifuge and separate serum or plasma as soon as possible after collection. Do not use hemolyzed samples.

Typically, the specimens may be refrigerated at 2–8°C for two weeks. For long term storage, they can be stored at –20°C. Avoid repeated freeze–thaw cycles. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed before analysis.

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** by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20–25°C).

PROCEDURE:

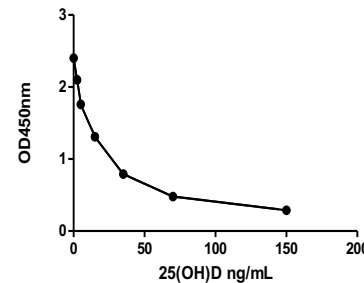
All reagents and specimens must be allowed to come to room temperature before use. All reagents must be GENTLY mixed without foaming. Once the procedure has started, all steps should be completed without interruption.

1. Dispense 10µl of 25-OH Vitamin D Standards, controls and samples into each well, as required.
2. Dispense 200µl 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).
4. **INCUBATION #1** – Incubate plate for 90 minutes at room temperature.
5. Briskly shake out the contents of the wells into a waste reservoir.
6. **WASH # 1** - Dispense 300µl 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 200µl 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 300µl 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.

Standard Curve:

Seven standard levels are included for each run. A typical standard curve is shown below.



25(OH)D (ng/ml)	Absorbance (450nm)
0	2.40
2.5	2.10
5	1.76
15	1.31
35	0.79
70	0.48
150	0.29

QUALITY CONTROL:

We recommend that each laboratory uses 25-OH Vitamin D controls to validate the performance of reagents.

RESULTS:

Results are expressed in ng/mL. Note: To convert to nmol/L, multiply results by 2.5. Example: 10ng/ml = 25nmol/L.

REFERENCE RANGE:

It is recommended that each laboratory establishes the range of normal values that corresponds to the population of their region.