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Product Information

25(OH) Vitamin D ELISA

Catalog Number **SE120139**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

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 forms: Vitamin D_2 and Vitamin D_3. Vitamin D_2 is obtained from dairy products; whereas, Vitamin D_3 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 D_2 and Vitamin D_3) are converted to 25-OH Vitamin D in the liver. The sum of the concentrations of 25-OH Vitamin D_2 and 25-OH Vitamin D_3 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]

The 25(OH) Vitamin D ELISA 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.

The concentration of Streptavidin-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 a 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 the amount of 25(OH) D in the sample. The assay measures both Vitamin D_2 and D_3. The total assay procedure run time is 2.5 hours.

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

Components

Materials Provided	96 Tests
Microwell plate coated with anti-Vitamin D	12 x 8 x 1
Vitamin D Standard Set: 7 vials (ready to use)	0.5 mL
Vitamin D Control Set: 2 vials (ready to use)	0.5 mL
Biotinylated 25(OH)D Reagent: 1 Vial (51x)	0.55 mL
Assay Diluent, 1 bottle	24 mL
Streptavidin-HRP, 1 bottle (ready to use)	23 mL
Stop Solution, 1 bottle (ready to use)	12 mL
TMB Substrate, 1 bottle (ready to use)	24 mL
Microplate sealing film	2
Wash Concentrate 20x, 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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

1. For serum, collect whole blood by venipuncture and allow clotting.
2. For plasma, mix the sample by gentle inversion prior to centrifugation.
3. Centrifuge and separate serum or plasma as soon as possible after collection.
4. Do not use hemolyzed samples.
5. The specimens may be refrigerated at 2–8 °C for two weeks. For long term storage, they can be stored at –20 °C.
6. Avoid multiple freeze-thaw cycles.
7. Prior to assay, the refrigerated or frozen-thawed samples must be allowed to equilibrate to room temperature for 30 minutes and mixed well.

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.
2. Equilibrate the needed volume of standards and reagents to room temperature before use.

51x Biotin conjugate:

Immediately before use, prepare 1x working solution by diluting 51-fold with Assay Diluent (e.g., Add 0.1 mL of the 50x Vitamin D-Biotin conjugate concentrate to 5 mL of Assay Diluent). Remaining Assay Diluent must be stored at 2–8 °C in dark and tightly capped.

20x Wash Buffer Concentrate

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 (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: 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 samples be run in duplicate.

Once the procedure has started, all steps should be completed without interruption.

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.

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Dispense 10 µL of 25(OH) D Standards, controls, and samples into each well, as required.
2. Dispense 200 µL of 1x working solution of biotinylated 25 (OH) D reagent 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. Incubate sealed plate for 90 minutes at room temperature (18–26 °C).
5. Carefully remove the seal on the plate and discard the contents of the wells.
6. Dispense 300 µL of 1x Wash Buffer into each well, and then discard the contents of the wells. Repeat twice for a total of 3 washes. Tap the wells on absorbent paper.
7. Dispense 200 µL of Enzyme Conjugate (Streptavidin-HRP) into each well and incubate for 30 minutes, at room temperature (18–26 °C).
8. Discard contents of the wells.

9. Dispense 300 µL of 1x Wash Buffer into each well, and then discard the contents of the wells. Repeat twice for a total of 3 washes. Tap the wells on absorbent paper.
10. Using a multichannel pipette, dispense 200 µL of TMB Substrate into each well.
11. Incubate for 30 minutes at room temperature, preferably in the dark.
12. Dispense 50 µL of Stop Solution into each well to stop the enzymatic reaction. Carefully mix plate contents for 20–30 seconds**.**
13. Read absorbance on ELISA Reader at 450 nm within 10 minutes of adding the Stop Solution.

Results

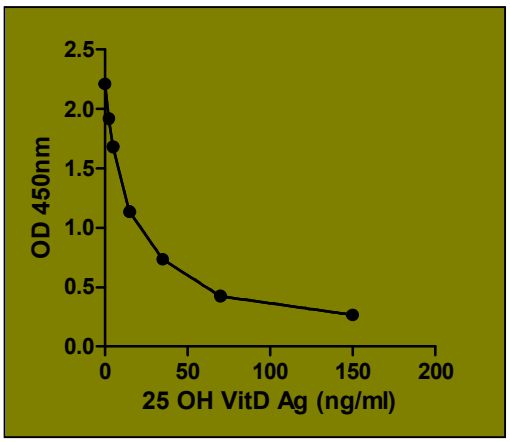
Results are expressed in ng/mL.

Note: To convert to nmole/L, multiply results by 2.5.

Example: 10 ng/ml = 25 nmole/L.

25(OH) Vitamin D (ng/mL)	Absorbance (450 nm)
0	2.214
1.25	1.920
2.5	1.683
10	1.137
35	0.737
70	0.425
150	0.267

A dose response curve can be established from the data by plotting standard concentration (abscissa) against corresponding absorbance (ordinate). Values for unknown samples are obtained by interpolation from the curve. The following data represent a typical dose/response curve.



References

1. Holick, M.F., Vitamin D Status: Measurement, Interpretation and Clinical Application. Ann. Epidemoil., **19**(2), 73-78 (2009).
2. Morris, H.A., Vitamin D: A Hormone for All Seasons-How Much is enough? Clin. Biochem. Rev., **26**, 21-32 (2005).
3. Bikle, D.D., Vitamin D and the skin. J. Bone Miner. Metab., **28**, 117-30 (2010).
4. Zerwekh, J.E., Blood biomarkers of vitamin D status. Am. J. Clin. Nutr., **87**, 1087S-91S (2008).
5. Moyad, M.A., Vitamin D: a rapid review. Dermatol. Nurs., **21**, 25-30 (2009).

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