

25-OH Vitamin D ELISA Test Kit

NAME AND INTENDED USE

Detection Kit for 25-OH Vitamin D (Enzyme-Linked Immunosorbent Assay, ELISA). It is used in quantitative tests for Vitamin D in human serum.

SUMMARY AND CLINICAL SIGNIFICANCE

Vitamin D is a fat-soluble steroid hormone precursor that is mainly produced in the skin by exposure to sunlight. Vitamin D is biologically inert and must undergo hydroxylation steps to become active. Our body can only synthesize vitamin D3. Vitamin D2 is taken up with fortified food or given by supplements. Physiologically, vitamin D3 and D2 are bound to the vitamin D-binding protein (VDBP) in plasma and transported to the liver to become 25-hydroxy vitamin D (25-OH vitamin D). As 25-OH vitamin D represents the major storage form, its blood concentration is used to assess the overall vitamin D status. More than 95 % of 25-OH vitamin D, measurable in serum, is 25-OH vitamin D3 whereas 25-OH vitamin D2 reaches measurable levels only in patients taking vitamin D2 supplements. Vitamin D is essential for bone health. In children, severe deficiency leads to rickets.

In elderly, the risk of falling has been attributed to vitamin D deficiency due to muscle weakness. Moreover, low 25-OH vitamin D concentrations are associated with lower bone mineral density. Insufficiency has also been linked to diabetes, cancer, cardiovascular disease, and autoimmune 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 deficiency 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.

The Vitamin D total assay employs special antibodies to capture both 25-hydroxyvitamin D3 and D2. This assay is intended for the quantitative determination of total 25-OH vitamin D in human serum and plasma, as an aid in the assessment of vitamin D sufficiency.

PRINCIPLE

The 25-OH Vitamin D Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one antibody for solid phase (microtiter wells) immobilization and another monoclonal antibody in the solutions. The third antibody which recognize the second antibody conjugated with an enzyme (horseradish peroxidase). In the assay procedure, standards and test specimen (serum) are added to the 25-OH Vitamin D antibody coated microtiter wells, incubation 30 minutes together with the second antibody. After wash, the third antibody labeled with horseradish peroxidase (conjugate) is added. If human 25-OH Vitamin D is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in forming sandwiches between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution. The color is changed to yellow and measured spectrophotometrically at 450 nm. The concentrations of Vitamin D is directly proportional to the color intensity of the test sample.

PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth.
2. Use only pipettes with disposable tips for each specimen.
3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8x12 wells), Ready to use. Monoclonal anti- 25-OH vitamin D -antibody coated microtiter plate and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2-8°C until expiration date.
2. HRP Conjugate: 1 vial of 22ml, Ready to use. Store at 2-8°C until expiration date.
3. Calibrator: 6 vials of 0.2ml. Labeled with S0 to S5 and the concentration of PRL is 0, 10, 20, 50, 100, 150 ng/ml. Store at 2-8°C until expiration date.
4. Control: 2 vials of 0.2ml.
5. Vitamin D Sample Diluent: 1 vial of 22 ml, Ready to use. Store at 2-8°C until expiration date.
6. TMB Substrate : 1 vial of 12 ml, Ready to use. Store at 2-8°C until expiration date.
7. Stop Solution: 1 vial of 6 ml, Ready to use. Store at 2-8°C until expiration date.
8. Wash buffer: 1 vial of 25ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2-8°C until expiration date.
9. Plate sealer: 2 pieces.
10. Plastic resealable bag: 1 set.
11. Instruction manual: 1 copy.

STORAGE OF TEST KITS AND INSTRUMENTATION

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, Dilute 25 ml of Wash Buffer (20x) into 475 ml of distilled water to prepare 500 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE:

1. Mark the microtiter strips to be used. All the Calibrators and controls should set duplicate.
2. Dispense 10µl of calibrators/controls/samples into respective wells. Gently but thoroughly mix for 10 seconds.
3. Dispense 200µl of Sample Diluent into each well, Mix gently for 30 seconds. It is very important to have a complete mixing in this setup. And incubation at room temperature for 30 minutes.
4. Wash each well for 5 times with about 0.3ml diluted washing buffer, 10 seconds each time.
5. Dispense 200µl of HRP Conjugate to each well.
6. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 30 minutes.

7. Wash each well for 5 times with about 0.3ml diluted washing buffer, 10 seconds each time.
8. Dispense 100µl of TMB substrate to each well.
9. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 15 minutes.
10. Dispense 100µl of stop solution to each well and mix completely.
11. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

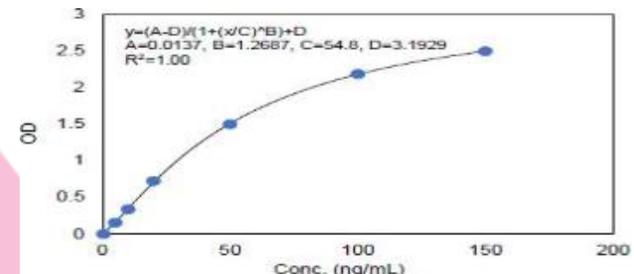
CALCULATION OF RESULTS

Calculate the mean absorbance value for each set of 25-OH Vitamin D reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng per ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of 25-OH Vitamin D in ng per ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against 25-OH Vitamin D concentrations shown in the X axis. Suggest: Use 4-Parameter standard curve to calculate sample values.

Vitamin D Values (ng/ml)	Absorbance (450nm)
0	0.056
10	0.231
20	0.507
50	1.141
100	1.866
150	2.327



This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.

EXPECTED VALUES

Deficiency: 0-10 ng/ml;
 Insufficiency: 10-30 ng/ml;
 Sufficiency: 30 to 100 ng/ml;
 Toxicity: >100 ng/ml.

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