Specificity

The compounds were tested for cross-reactivity using the Abraham method with 25(OH)D2, 25(OH)D3 cross reacting at 100%; 1, 25(OH)$_2$D3 at 8.3%; 3-epi-25 (OH)D3 at 66%; Vitamin D2 at <1.0% and Vitamin D3 at <1.0%.

References: Holick MF (2007) N Engl. J. Med. 357, 266-81; Gordon NP et al. (2012) Nutrition J. 11, 104-113.

ELISA kits available from ADI (see details at the web site)

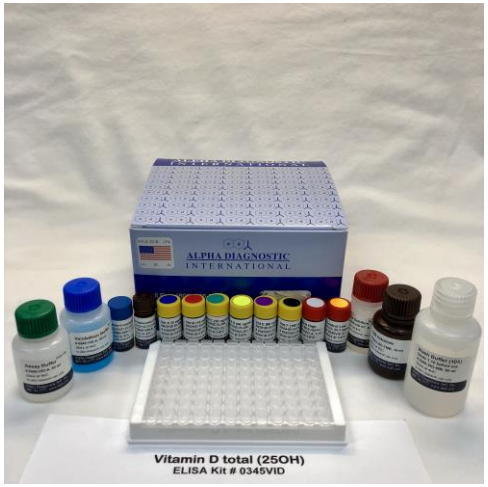
#0010	Human Leptin				
#200-120-AGH	Human globular Adiponectin (gAcrp30)				
#0700	Human Sex Hormone Binding Glob (SHBG)				
#0900	Human IGF-Binding Protein 1 (IGFBP1)				
#1000	Human C-Reactive Protein (CRP)				
#1190	Human Serum Albumin		#1200	Human Albumin (Urinary)	
#1750	Human IgG (total)		#1760	Human IgM	
#1800	Human IgE	#1810	Human Ferritin		
#1210	Human Transferrin (Tf)		#0020	Beta-2 microglobulin	
#1600	Human Growth Hormone (GH)				
#0030	Human Insulin	#0040	Human C-peptide		
#0100	Human Luteinizing Hormone (LH)				
#0200	Human Follicle Stimulating Hormone (FSH)				
#0300	Human Prolactin (PRL)				
#0400	Human Chorionic Gonadotropin (HCG)		#0410	HCG-free beta	
#0600	Human Thyroid Stimulating Hormone (TSH)				
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)		
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)		
#1850	Human Cortisol		#1860	Human Progesterone	
#1865	Human Pregnolone		#1875	Human Aldosterone	
#1880	Human Testosterone		#1885	Human free Testosterone	
#1910	Human Androstenedione		#1920	Human Estradiol	
#1925	Human Estrone		#1940	Dihydrotestosterone (DHT)	

Instruction Manual No. M-0345-VID

Vitamin D total (25OH) ELISA kit

Cat # 0345-VID, 96 Tests

For Quantitative determination of Vitamin D total (25OH) in Human Serum and Plasma



For In Vitro Research Use Only

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Vitamin D total (25OH) ELISA KIT # 0345-VID

ELISA Kit for the quantitative determination of Vitamin D total (25OH) in Human Serum and Plasma. : Kit Contents (96 tests):

Kit Components	96 tests
Anti-25 (OH) Antibody coated strip plate (96 wells), #0345VID-1	1 plate
25(OH)D Standards (A-F), #0345VID-2A-F (0, 10, 20, 40, 80 & 160 ng/ml), 1 ml/vial	6 Vials
25(OH)D Control Low 1 ml, # 0345VID-3	1 Vial
25(OH)D Control High 1 ml, # 0345VID-4	1 Vial
25(OH)D Incubation buffer 20 ml, #0345VID-5, blue cap	1 Bottle
25(OH)D Assay buffer 20 ml, #0345VID-6, green cap	1 Bottle
25(OH)D **Biotin- Conjugate Concentrate, 100x** 1 ml, #0345VID-7, blue cap	1 Vial
HRP-**Streptavidin Conjugate concentrate, 100x** 0.3 mL, #0345VID-8, brown cap	1 Vial
Wash buffer (10X) , #0345VID-WB, 50 ml	1 Bottle
TMB substrate, # 0345VID-TMB, 16 ml	1 Bottle
Stop solution, 6 ml # 0345VID-ST, red cap	1 Bottle
Instruction Manual, M-0345-VID	1

Intended Use

Vitamin D total (25OH) ELISA kit is used for the quantitative detection of Vitamin D total (25OH) in human Serum and Plasma.. For in vitro research use only (RUO).

Introduction

Vitamin D is the generic term used to designate Vitamin D2 or ergocalciferol and Vitamin D3 or cholecalciferol. Humans naturally produce Vitamin D3 when the skin is exposed to ultraviolet sun rays. In the liver mainly, Vitamin D3 is metabolized into 25-Hydroxyvitamin D3 (25OH D3) which is the main form of Vitamin D circulating in the body. 25OH D3 is a precursor for other Vitamin D metabolites and has also a limited activity by itself. The most active derivative is 1,25-hydroxyvitamin D3, produced in the kidney (or placenta) by 1-hydroxylation of 25OH D3. 25OH Vitamin D stimulates the intestinal absorption of both calcium and phosphorus and also bone resorption and mineralization. 25OH Vitamin D might also be active in other tissues responsible for calcium transport (placenta, kidney, mammary gland …) and endocrine gland (parathyroid glands, beta cells…). Vitamin D3 and Vitamin D2 are also available by ingestion through food or dietary supplementation. As Vitamin D2 is metabolized in a similar way to Vitamin D3, both contribute to the overall Vitamin D status of an individual. It is the reason why it is very important to measure both forms of 25OH Vitamin D equally for a correct diagnosis of Vitamin D deficiency, insufficiency or intoxication. Vitamin D deficiency is an important risk factor for rickets, osteomalacia, senile osteoporisis, cancer and pregnancy outcomes. The measurement of both 25OH Vitamin D forms is also required to determine the cause of abnormal serum calcium concentrations in patients. Vitamin D intoxication has been shown to cause kidney and tissue damages.

For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

3. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, ADI shall have no liability.

4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

PERFORMANCE CHARACTERISTICS

Sensitivity

Limit of Detection (LoD) = 5.5 ng/mL of 25(OH)D

Interference

The following substances were tested and did not show significant interference in the assay up to concentrations more elevated than the highest occurring levels: hemoglobin up to 7.5 mg/mL; bilirubin conjugated and free up to 200 µg/mL; triglycerides up to 5.5 mg/mL; cholesterol up to 2.6 mg/mL; ascorbic acid up to 10 mg/mL; biotin up to 40 µg/mL and caffeine up to 10 µg/mL.

Linearity

Serum 83-103%

Precision

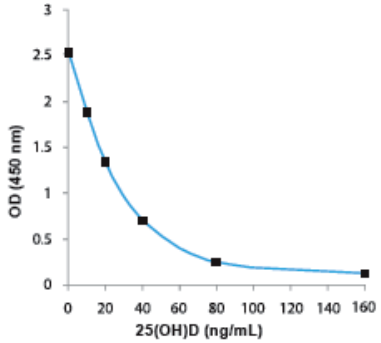
Intra-Assay CV% <3-7%

Inter-Assay CV% <4-9%

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A450nm
A1, A2	Std. A (0 ng/ml)	2.53
B1, B2	Std. B (10 ng/ml)	2.25
C1, C2	Std. C (20 ng/ml)	1.97
D1, D2	Std. D (40 ng/ml)	1.45
E1, E2	Std. E (80 ng/ml)	0.71
F1, F2	Std. F (160 ng/ml)	0.13
G1, G2	Sample 1	1.83

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



Calculation of results:

A dose response curve is used to ascertain the concentration of Vitamin D total (25OH) in unknown specimens.

1. Record the A450 obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the A450 for mean duplicate calibrator versus the Vitamin D total (25OH) concentration in pg/ml on linear graph paper. Connect the points with a best-fit curve.
3. To determine the concentration of Vitamin D total (25OH) for an unknown, locate the average A450 of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.83) intersects the dose response curve at 18.57 ng/ml Vitamin D total (25OH) concentration (See Figure 1).

Interpretation

1. *Measurements and interpretation of results must be performed by a skilled individual or trained professional.*
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
   The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.

PRINCIPLE OF THE TEST

Vitamin D total (25OH) ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Anti-25 (OH)D Antibody. During the reaction, unlabeled 25(OH)D present in the sample, control or standard dissociates and binds to the anti-25(OH)D antibody immobilized on the microplate wells. After washing and during next incubation, complex of 25(OH) D-biotin conjugate and streptavidin-HRP conjugate competes with antibody-bound 25(OH)D for antibody binding sites. Excess conjugate and unbound sample or standard are washed from the plate. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of diluted sulphuric acid solution (stop solution) and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of 25(OH)D in the samples is then determined by comparing the OD of the samples to the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (50-200 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

SPECIMEN COLLECTION AND STORAGE

The specimens shall be blood, serum in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) with no anti-coagulants. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8oC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20oC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml (50µl) of the specimen is required.

Notes:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from ADI's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance**.**

REAGENTS PREPARATION FOR THE ASSAY:

Wash Buffer (10X): Dilute the wash buffer 1:10 with distilled water (If the whole plate is to be used dilute 50 mL of wash buffer concentrate in 450 mL deionized or distilled water). Store diluted wash buffer at 2-8oC.

Preparation of Working Conjugate Solution: To a disposable tube or glass bottle, first add required amount of assay buffer and then to this add a 1:100 volume of 25(OH)D-Biotin conjugate Concentrate and a 1:100 volume of Streptavidin-HRP Conjugate concentrate. (For example: if the whole plate is to be used, to 16 mL of assay buffer in a glass test tube or bottle, add 0.16 mL of 25(OH)D-Biotin conjugate Concentrate and 0.16 mL of Streptavidin-HRP Conjugate concentrate)

Mix the working conjugate thoroughly and store in a dark place until it is used in step 5 of assay procedure.

Note: It's very important to add assay buffer to glass tube first and then add the conjugate concentrates to assay buffer. Failure to prepare in this order can lead to decreased OD values. Prepare working conjugate solution just before starting assay procedure. The working conjugate solution is stable for up to 4 hours, therefore it can be prepared between 0 and 120 minutes before starting the assay.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. Do not freeze and thaw.

Quality Control:

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE, 25-28oC, BEFORE USE). Prepare working solutions of conjugate and wash buffer (see page 3). Bring all reagents and solutions to room temp. (25-28oC).*

1. Organize the microplates wells for each serum reference standard, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette **25 µl of standard, control and serum or plasma Sample** into the assigned wells in duplicate.
3. Pipette 150 ul of incubation buffer into each well. Mix gently and incubate for 60 minutes at room temp in a dark place (no shaking).
4. Wash plate 3 times with 300 ul/well of diluted wash buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
5. Pipette 1**50 µl of the working conjugate solution** into each well.
6. Mix the microplate gently for 20-30 seconds by gentle tapping against the palm. **Incubate for 30 minutes** at room temp in a dark place (no shaking).
7. Wash plate **3 times** with 300 ul/well of diluted wash buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
8. Add **150 µl of TMB substrate** reagent to all wells. Note: Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.
9. **Incubate for** 10-15 minutes at room temp in a dark place (no shaking)**..**
10. Add **50 µl of stop solution** to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
11. **Read the absorbance in each well at 450nm**. The results should be read within fifteen (15) minutes of adding the stop solution.

Note: 25(OH)D Control Low Acceptable range: 14.0 - 23.3 ng/mL
25(OH)D Control High Acceptable range: 35.2 - 58.6 ng/mL