GENLISA™ 25OH Vitamin D ELISA

REF : KBD501

Ver 2.0

IVD

Enzyme Immunoassay for the Quantitative Determination of 25OH Vitamin D in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBD501 Σ 96 tests

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Introduction:

25OH Vitamin D is a commonly collective term for a family of closely related seco-steroids. Upon exposure to sunlight, 7-dehydro-cholesterol, located deep in the actively growing layers of the epidemis, undergoes photolytic cleavage of the “B” ring to yield pre-vitamin D3 which is isomerized to vitamin D3 (cholecalciferol). Vitamin D3 and vitamin D2 (ergocalciferol) may also be obtained by dietary supplementation or from a limited number of foods. Vitamin D2 is metabolized in a similar way to vitamin D2.

25OH Vitamin D is stored in adipose tissue and enters the circulation bound to 25OH Vitamin D binding protein (VDBP) and albumin. In the liver, 25OH Vitamin D is hydroxylated to give 25OH Vitamin D which also circulates as a complex with VDBP. A small proportion of the 25OH Vitamin D is further hydroxylated in the kidney, under direct regulation by parathyroid hormone and ionized calcium levels, to form the biologically active calcitropic hormone 1, 25 di-hyroxyvitamin D. Further hydroxylation and metabolism of 25OH Vitamin D produces compounds that are water soluble and readily excreted.

Hepatic 25OH Vitamin D activity is not tightly regulated, and changes in cutaneous activity is not tightly regulated, and changes in cutaneous production of vitamin D3, or ingestion of 25OH Vitamin D (D3 or D2) will result in changes in circulating levels of 25OH Vitamin D.

Serum concentration of 25OH Vitamin D is considered to be the most reliable measure of overall 25OH Vitamin D status and thus can be used to determine whether a patient is 25OH Vitamin D sufficient. Assessment of 25OH Vitamin D status may be required to determine the cause of abnormal serum calcium concentration in patients.

Intended Use:

The GENLISA™ 25OH Vitamin D ELISA is intended for the quantitative determination of 25OH Vitamin D in human serum and plasma.

Principle:

25OH Vitamin D GENLISA™ ELISA method is a quantitative determination based on enzyme-linked immunosorbent assay (ELISA) to determine the level of 25OH Vitamin D and other hydroxyrelated metabolites in serum or plasma. Standards, Controls and Samples are diluted with biotin labelled 25-OH Vitamin D solution. The diluted samples are incubated in microtitre wells which are coated with a highly specific sheep 25-OH Vitamin D antibody for 2 hours at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labeled avidin, is added and binds selectively to complexed biotin and, following a further wash step, color is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microtitre plate reader, color intensity developed being inversely proportional to the concentration of 25-OH Vitamin D.

Materials Provided:

1. Anti-25OH Vitamin D Coated Microplate Plate (8x12 wells) - 1 no
2. 25OH Vitamin D Standards (lyophilized, the exact concentration of each standard is indicated on the vial label) - 7 nos
3. High Control (lyophilized, the range is indicated on the vial label) - 1 ml
4. Low Control (lyophilized, the range is indicated on the vial label) - 1 ml
5. (50X) Biotinylated 25OH Vitamin D (concentrated, lyophilized) – 1 Vial
6. Biotin Antigen Dilution Buffer - 50 ml
7. Enzyme Conjugate - 22 ml
8. (20X) Wash Buffer - 50 ml
9. TMB Substrate - 28 ml
10. Stop Solution - 13 ml
11. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.
4. Reconstitute 25OH Vitamin D Biotin solution can be stored at 2-8°C for up to 8 weeks. The 25OH VitaminD Biotin solution must be stored as above in the dark immediately after use.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Note: Do not use sera preserved with sodium azide or thiomersal. Store samples at 2–8 °C within 72 hours or at -20°C for longer. Do not freeze/thaw more than once.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. **Biotin Working Solution:** Add 3 ml of **Biotin Antigen Dilution Buffer** to the **Biotinylated (50X) 25OH Vitamin D** (blue color) Solution. Replace the stopper and stand for 10-15 minutes at Room Temperature. Invert several times to ensure complete reconstitution and thorough mixing. Add the **reconstituted Biotinylated (50X) 25OH Vitamin D** (3 ml) back into the remaining **Biotin Antigen Dilution Buffer**. Mix well by inversion. Store at 2-8°C in the dark.
3. **Standards/Control/Sample Preparation:** Add **10 ul** of **each Standard, Controls and Samples** in the appropriately labeled tubes, add **0.4 ml** of Biotin Working solution to all the tubes, vortex thoroughly for 10 seconds.
4. **(1X) Wash Buffer Dilution**: To make **(1X) Wash Buffer**, add 50 ml of **(20X) Wash Buffer** to 950 ml of **DI water**. This is the working solution

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add **200ul** of **diluted Standards, Controls** and **Samples** into each well

3. Cover the plate with plate sealer and **Incubate** for **18-25°C** for **2 hours**
4. Aspirate and wash plate 3 times with **(1x) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any Residue can interfere in the reading step. All the washes should be performed similarly.
5. Add **200 ul Enzyme Conjugate** to all wells.
6. Cover the plate with plate sealer and **Incubate** for **18-25°C** for **30 minutes**.
7. Repeat the Wash Step as mentioned in step 4.
8. Add **200 ul** of **TMB Substrate** to all wells.
9. Cover the plate with plate sealer and Incubate for 18-25°C for 30 minutes.
10. Add **100 ul** of **Stop Solution**.
11. Read absorbance on ELISA Reader at 450 nm within 30 minutes of adding the Stop Solution

Calculations of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown 25OH Vitamin D concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the 25OH Vitamin D Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4-PL or a polynomial curve (2nd order) is best recommended for automated results.

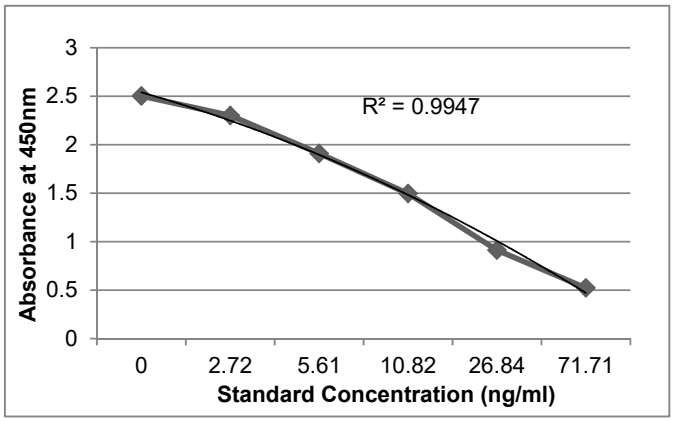
Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods.

Typical Data

Standard (ng/ml)	Abs1	Abs2	Mean Abs	Interpolated Concentration	% Interpolated against actual Concentration
0	2.476	2.530	2.503	-	-
2.72	2.313	2.288	2.301	2.29	84.2
5.61	1.912	1.908	1.91	5.90	105.4
10.82	1.495	1.499	1.497	10.99	101.8
26.84	0.919	0.905	0.912	26.04	97.2
71.71	0.521	0.522	0.522	67.84	94.7
152.24	0.372	0.368	0.37	183.67	120.8

Note the Standard / Calibrator concentrations will differ lot to lot and request to check the concentrations on the label with the standards provided in the kit. This is a typical data only and is not to used for reporting any samples in your laboratory.

Typical Graph



Validity of the Test:

The test is valid if following conditions are met.

Low Control: 9.9 – 18.4 ng/ml
High Control: 39.5 – 64.4 ng/ml

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:

Sensitivity:
Limit Of Quantification: It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be less than 2.5 ng/ml

Specificity:
The monoclonal antibodies used in the kit are specific for 25OH Vitamin D.

Expected Normal Value:

Level	Range
Deficiency	<10.0 ng/ml
Insufficiency	10 ng/ml - 29.9 ng/ml
Sufficiency	30 ng/ml - 100 ng/ml
Toxicity	> 100 ng/ml

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.

- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

THANK YOU FOR USING KRISHGEN PRODUCT!

Krishgen Pudgala LLP

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **10 ul Samples, Standards and Controls** into each well.
3	Add **200 ul Biotin Working Solution** into each well.
4	Shake gently for 20 seconds to mix well. **Incubate** at **37ºC** for 90minutes.
5	Aspirate and **wash plate 5 times** with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6	Add **200 ul Streptavidin:HRP Conjugate** to all wells. Shake gently for 30 seconds. Incubate at 37ºC for 30 minutes
7	Repeat the Aspiration / Wash Step as mentioned in step 5.
8	Add **100 ul** of **TMB Substrate** to all wells. Incubate for **37ºC** for 10minutes.
9	Add **100 ul** of **Stop Solution**. **Read result with an ELISA reader at 450 nm** within 10 minutes of stopping the reaction.

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
STD	**25OH Vitamin D Standards**
CTRL	**Control**
ASSY DIL	**Assay Diluent**
BIOTIN AB	**Biotin labelled Antibody**
HRP CONJ	**Conjugate Horseradish Peroxidase**
40X WASH BUF	**(40X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**