25OH Vitamin D GENLISA™ ELISA

REF : KBD501

Ver 4.0

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** 

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Introduction:

25-hydroxy vitamin D (25-OH Vit D), also known as calciferol, is a steroid-like, fat soluble pro-hormone which has two main forms: D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D is one of the necessary elements for maintaining proper bone mineral density by enhancing intestinal calcium and phosphate absorption and reducing renal excretion. Many studies have reported that some human genes related to cell growth are regulated by the Vitamin D and sufficient vitamin D values may reduce the incidence of cancer. Vitamin D is synthesized in the skin by exposure to sunlight and also obtained from the diet mainly from grain products, fatty fish such as, sardines, liver oils, egg yolks, and milk. Sever Vitamin D deficiency is an important risk factor resulting in delayed growth and rickets in children as well as osteomalacia in adults. Insufficient levels of vitamin D are associated with subclinical deficiencies and diseases including, secondary hyperparathyroidism, abnormal cell metabolism, impaired immunity, bone fracture and osteoporosis. Continuous vitamin D supplementation results in Vitamin D toxicity and hypercalcaemia which leads to soft- tissue calcium deposition.

Intended Use:

The 25OH Vitamin D ELISA kit is designed for the quantitative detection of 25OH Vitamin D in human serum or plasma samples. It is intended as an aid to the diagnosis of certain systemic autoimmune rheumatic diseases. This kit is for in vitro diagnostics (IVD) for professional use only.

Principle:

The test principle is based on competitive inhibition ELISA technique. This technique utilizes anti-vitamin D monoclonal antibody (mAb Anti-25-OH vitamin D) which is coated in microtiter wells. Patient sera and standards are added following with a certain amount of extraction buffer to release vitamin D from its binding protein (DBP-complex). After the first incubation and washing step, a constant amount of biotinylated 25-OH vitamin D and HRP-conjugated streptavidin are added simultaneously to the wells. The added reagents, which are in the complex form (Biotinylated 25-OH vitamin D bound to streptavidin-HRP), competes with the endogenous serum vitamin D for a limited number of binding sites on the coated anti vitamin D antibodies. After incubation the wells are completely washed to remove unbound reagents and a solution of chromogen-substrate is added and incubated for 15 minutes, resulting in the development of a blue color. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

The color intensity is proportional to the amount of biotinylated 25-hydroxy vitamin D and is inversely related to the amount of endogenous 25-OH vitamin D in the test sample. By reference to a series of vitamin D standards assayed in the same way, the concentration of 25-OH vitamin D in the unknown sample is quantified.

Materials Provided:

1. Anti-25-OH Vitamin D Microtiter Coated Plate (8x12 wells) - 1 no
2. 25-OH Standards (0,5,10,30,60,120 ng/ml) – 6 vials/1ml
3. Assay Buffer – 7.5 ml
4. Extraction Buffer – 7.5 ml
5. (20X) Enzyme Conjugate (concentrated) – 0.75 ml
6. Conjugate Diluent Buffer – 15 ml
7. Low Control–1 ml
8. High Control – 1 ml
9. (20X) Wash Buffer – 50 ml
10. TMB Substrate - 12 ml
11. Stop Solution - 12 ml
12. 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 pipette to measure volumes ranging from 10 ul, 100 ul to 1000 ul
3. Deionized (DI) water
4. Distilled Water

5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.

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, re-centrifuge.

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, re-centrifuge.

Test Sample Preparation:
If the sample is more concentrated, dilute it as needed.
Note: Control sera are ready to use and do not need any dilution.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 50 ml of Wash Buffer (20X) to 450 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 **20 ul Standard, Low Control, High Control and Samples** in appropriate wells.

3. Add 100 ul Assay Buffer into each well. In order to prepare the Reaction buffer per well, add 75 µl of Extraction buffer and 75 µl of assay buffer into a tube and mix well.

4. Shake the microplate gently for 2 minutes to mix the contents. Then cover the microtiter wells with cardboard sealer. Incubate it for 45 minutes at 37ºC incubator.

5. Add **100 ul** of **Enzyme Conjugate** to each well except blank well.

6. Cover the microtiter wells with cardboard sealer and incubate it for 15 minutes at 37ºC incubator.

7. 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.

8. Add **100 ul** of **TMB Substrate** into each well.

9. Incubate at room temperature for 15 minutes.

10. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 30 minutes of stopping the reaction.

Calculation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Use the mean absorbance value for each sample; determine the corresponding concentration of 25-OH Vitamin-D in ng/ml from the standard curve.

Low Control Range: 5.0 – 19 ng/ml
High Control Range: 30 – 80 ng/ml

Results of a typical standard curve of a 25-OH-Vitamin-D ELISA are shown in table 1.

Table 1: Example of Standard Curve

	Standards (ng/ml)	**Mean Absorbance (450/630 nm)**
Standard1	0	2.50
Standard2	5	1.26
Standard3	10	0.97
Standard4	30	0.58
Standard5	60	0.34
Standard6	120	0.18

Expected Values:

Each laboratory must establish its own normal range based on patient population. Based on a limited number of healthy adult blood specimens, the mean 25-OH-vitamin-D concentrations are shown in the below table.

Level	**Range (ng/ml)**
Deficient	<20
Insufficient	20-29
Sufficient	30-100
Potential Toxicity	>100

Validity of the Test

The assay is considered valid if:
1. The OD (450 nm) of calibrator higher than 0.17.
2. The OD (450 nm) of positive control higher than 1.2 and for the negative control less than 0.1.

Performance Characteristics:

1. **Sensitivity**
   The sensitivity of 25-OH-Vitamin-D kit is 3.5 ng/ml and values less than this amount should be reported as <3.5 ng/ml.

2. **Test Precision**

Intra, as well as Inter-assay precision, carried out by 3 different sera, and the results are shown in tables 1 and 2:

Table 1: Intra-assay:

No.	No. of tests*	Mean (ng/ml)	SD	CV %
1	10	12	1.7	14.2
2	10	54	2.21	4.1
3	10	104	2.28	2.2

Table 2: Inter-assay:

No.	No. of tests*	Mean (ng/ml)	SD	CV %
1	24	10.26	1.71	16.7
2	24	33.26	3.66	11
3	24	112.00	3.80	3.4

3. Specificity:

The specificity of this 25-OH-Vitamin-D ELISA test was determined by adding various Vitamin D metabolites to serum samples. The results of cross-reactions are shown in table below:

Cross-reactants	Concentration (ng/ml)	Cross-reaction (%)
25-OH-Vitamin-D3	10	100
25-OH-Vitamin-D2	10	100
1, 25-OH-Vitamin-D3	100	9.5
1, 25-OH-Vitamin-D2	70	6
Vitamin-D3	25	<0.1
Vitamin-D2	20	<0.5
24, 25-OH-Vitamin-D3	50	3.5
3-epi-25-OH-Vitamin-D2	20	5
3-epi-25-OH-Vitamin-D3	20	1.5

4. Recovery:

Certain amounts of 25-OH-Vitamin-D were added into 4 different sera with known concentrations of 25-OH-Vitamin-D and their recovery were determined. The results are shown in below table:

Sr. No.	25-OHVitamin-D level in serum (ng/ml)	Added 25-OHVitamin-D level (ng/ml)	Expected value (ng/ml)	Observed spike value (ng/ml)	Recovery (%)
1	15	10	12.5	11.9	95.2
1	15	25	20	19	95
1	15	70	42.5	40.5	95.2
2	38	10	24	25	104.1
2	38	25	31.5	32	101.5
2	38	70	54	56.5	104.6
3	59	10	34.5	33.5	97.1
3	59	25	42	40	95.2
3	59	70	64.5	62	96.1
4	104	10	57	59.5	104.4
4	104	25	64.5	66	102.3
4	104	70	87	91	104.6

5. Linearity:

To verify test linearity, five different serum samples with known concentration of 25-OH-Vitamin-D were diluted with standard zero (st.0) and tested by this ELISA kit. The results are shown in the below table:

Sr. No.	25-OH-Vitamin-D undiluted specimen (ng/ml)	Recovery (%)			
		1:2	1:4	1:8	1:16
1	18.3	110	108	*	*
2	37.3	92	93	97	*
3	58.9	98	111	92	89
4	95	103	106	108	115
5	112	104	103	99	116

* **Not applicable (below the detection limit).**

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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!

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EC REP

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

** Under CDSCO Registration, please note*

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **20 ul Standard, Low Control, High Control and Samples** in appropriate wells.
3	100 ul Assay Buffer into each well. In order to prepare the Reaction buffer per well, add 75 µl of Extraction buffer and 75 µl of assay buffer into a tube and mix well.
4	Shake the microplate gently for 2 minutes to mix the contents. Then cover the microtiter wells with cardboard sealer. Incubate it for 45 minutes at 37ºC incubator.
5	Add **100 ul** of **Enzyme Conjugate** to each well except blank well.
6	Cover the microtiter wells with cardboard sealer and incubate it for 15 minutes at 37ºC incubator.
7	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.
8	**100 ul** of **TMB Substrate** into each well.
9	Incubate at room temperature for 15 minutes.
10	Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 30 minutes of stopping reaction.

SYMBOLS KEY

MTP	**Microtiter Plate (8x12 wells)**
STD	**Standards**
ENZY CONJ	**Enzyme Conjugate**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**