Vitamin D Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAVTD1	Vitamin D Elisa	96 Tests

Intended Use:

The AMS Vitamin D Elisa assay is intended to be used for the quantitative determination of Vitamin D in human serum.

Summary and Principle:

Vitamin D is a steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. It is available in two major forms Vitamin D3 and Vitamin D2. (cholecalciferol and ergocalciferol). Vitamin D2 is less active than Vitamin D3. Vitamin D3 is produced in the skin after exposure to ultraviolet light. Vitamin D deficiency has been linked to many diseases including osteoporosis, rickets, osteomalacia, cancers and cardiovascular diseases. 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 vitamin D concentrations from reaching toxic levels.

This Elisa assay used a solid phase of coated wells bound with anti-Vitamin D antibodies. When incubated with Vitamin D standards, controls and samples and the Vitamin D biotin conjugate at room temperature the biotin labelled vitamin D competes with the endogenous Vitamin D for binding sites on the coated anti-Vitamin D antibodies. After a bound free separation step, HRP labelled Streptavidin is added, which binds to the biotin labelled vitamin D. Unbound HRP streptavidin is removed by a wash step and a substrate reagent is added thereafter. This results in a blue colour and after stopping the reaction using the stop solution, the wells are measured for their absorbances using 450nm. The colour intensity is inversely proportional to the concentration of vitamin D.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells	Each microwell is coated with anti-Vitamin D antibodies. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C.
Vitamin D Calibrators	7x0.25ml	7 vials containing Vitamin D at concentrations of 0.0, 1.25, 2.5, 10, 35, 70 and 150 ng/ml made up in a human serum matrix. The Calibrators are ready to use. Stable up to expiry date when stored at 2-8°C without contamination.
Vitamin D Control Set	2x0.25ml	2 vials containing Vitamin D at concentrations provided in the label. The controls are ready to use and are stable up to expiry when stored at 2-8°C without contamination.
Biotinylated Reagent (51X)	1x0.5ml	1 vial containing 0.5ml of Concentrated Biotinylated Vitamin D antibodies in Buffered saline. Reconstitute the required amount immediately before testing. Store remaining at 2-8°C.
Assay Diluent	1x24ml	Diluent for the Biotinylated Reagent. Store at 2-8°C.
Streptavidin HRP Solution	1x23ml	HRP labelled Streptavidin. Store at 2-8°C.
Wash Buffer Concentrate	1x25ml	PBS-Tween at pH 7.4. 50X concentrate. Once open, stable for one month at 2-8°C. The concentrate must be diluted with 475ml of distilled water. Once diluted it is stable between 18 – 24°C for two months.
Substrate Solution	2x12ml	TMB Substrate in buffer. Ready to use. Store at 2-8°C.
Stop Solution	1x12ml	Diluted HCl(1N) Ready to use. Once open, stable for 2 months at 2-30°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials provided but not required:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum, heparinised plasma or EDTA plasma samples can be used with this assay.

- For serum, collect whole blood by venepuncture and allow clotting.
- For plasma, mix the sample by gentle inversion prior to centrifugation.

Centrifuge and separate serum or plasma as soon as possible after collection. Do not use haemolysed samples.

The specimens may be refrigerated at 2-8°C for two weeks. For long term storage, they can be stored at -20°C. Avoid repeated freeze-thaw cycles. Allow the refrigerated or frozen thawed samples to equilibrate to RT for 30 minutes before use; samples must be mixed before analysis.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labelled expiry date, whichever is earlier.

Precautions and Safety:

The Elisa assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify them.

1. **For professional use only.**
2. **Follow the instructions in this IFU as reliability of results cannot be guaranteed if there are deviations from the instructions.**
3. **The calibrators contain human source components which have been tested and found non-reactive for Hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B or other infectious agents are absent, these reagents should be handled at the Biosafety level 2, as recommended in the CDC/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.**
4. **The kit is intended for Research use only. Not for use in diagnostic procedures.**
5. **It is recommended that the calibrators, controls and serum samples be run in duplicate.**
6. **Do not pipette by mouth. Do not smoke, eat, drink in the areas in which specimens or kit reagents are handled.**
7. **The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.**
8. **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.**

Procedure:

Reagent preparation:

1. Standards and Reagents: Standards are serum based solutions are stable when stored at 2-8°C, protected from light and contamination until the expiry date on the label. Equilibrate the required volume of standards and reagents to room temperature before use.
2. **Wash Buffer**: Prepare diluted wash buffer by adding the contents of the bottle of wash buffer (25ml) to 475ml of distilled water. This diluted wash buffer is stable for 2 months at 2-30°C.
3. **Biotinylated Reagent** : IMMEDIATELY BEFORE USE, prepare a working solution of Biotinylated Reagent by diluting the reagent with the Assay diluent in the ratio 1:51. For example: to 0.1ml of the Biotinylated reagent concentrate add 4.9ml of Assay Diluent. Store the remaining Assay Diluent at 2-8°C in dark.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series. Once this procedure is started, all steps must be completed without interruption.

STEP 2

Addition of Samples and calibrators: Add 10ul of Calibrators, Controls and Samples to each well.

STEP 3

Addition of Diluted Biotinylated Reagent: Add 200ul of the Biotinylated Reagent into each well. Carefully mix the contents of each well for 20 seconds using a plate shaker at 200-400 rpm. Remove from shaker and cover the plate with the adhesive plate seal making sure that there is complete seal over each well.

STEP 4

Incubation: Incubate for 90 minutes at Room temperature.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 3 times with diluted washing buffer of 300ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of HRP Streptavidin solution: Add 200ul of HRP Streptavidin Solution to each well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at room temperature.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 3 times with diluted washing buffer of 300ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 9

Addition of Substrate: Using a multichannel pipette dispense 200ul of the Substrate solution into each well.

STEP 10

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at room temperature.

STEP 11

Addition of Stop Solution: Dispense 50ul of Stop solution into each well to stop the Enzymatic reaction. Carefully mix the plate for 20-30 seconds.

STEP 12

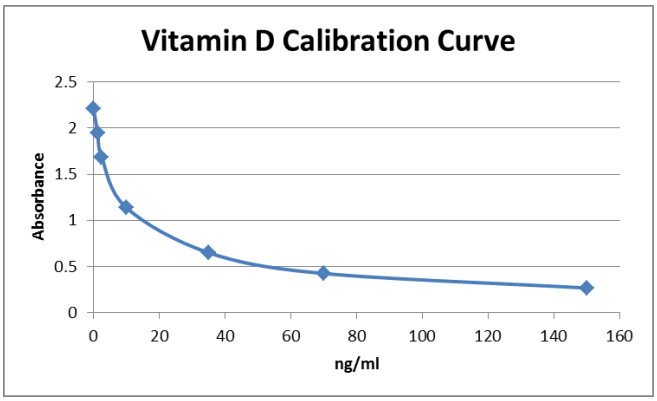
Measurement: Read the absorbance of the wells at 450nm using a microplate reader within 10 minutes of adding the Stop solution. Note down the absorbances.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in ng/ml in X axis.
- Draw a point to point (best fit) curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	2.214	0.0 ng/ml
CAL B	1.920	1.25 ng/ml
CAL C	1.683	2.50 ng/ml
CAL D	1.137	10.0 ng/ml
CAL E	0.737	35.0 ng/ml
CAL F	0.425	70.0 ng/ml
CAL G	0.267	150.0 ng/ml



Performance Characteristics:

Character	Actual
Intra Assay Variation	<6%
Total Imprecision	<8%
Limit of Quantitation	1.25 ng/ml
Spike and Recovery	98.7 %
Dilution Studies	98.4%
Specificity 25 Hydroxy Vitamin D3	100%
Specificity 25 Hydroxy Vitamin D2	122%
Vitamin D2 or D3	<0.1%

Method Comparison:

In an independent study this assay showed excellent correlation with LC/MS/MS R^2 = 0.949; N=20.

Reference range:

It is recommended that each laboratory establish its own normal reference ranges for the population that it serves.

References:

1. Holick, MF. Vitamin D Status: Measurement, Interpretation and Clinical Application. Ann.Epidemiol. 2009, 19(2):73-78.
2. Morris H.A. Vitamin D: A Hormone for All seasons – How much is enough? Clin. Biochem Rev., 2005, 26, 21-32.
3. Bikle D.D. Vitamin D and the Skin. J.Bone Miner.Metab 2010, 28, 117-30.
4. Moyad M,A. Vitamin D: a rapid review. Dermatol Nurs., 2009, 21, 25-30.

REF Cat No. LOT Lot Number Manufacturer

Manufactured Consult IFU

Expiry Store @ 2-8 °C

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