Datasheet for ABIN1774788

25-OH Vitamin D ELISA Kit

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Overview

Quantity:	96 tests
Target:	25-OH Vitamin D
Reactivity:	Human
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	The kit is a solid phase enzyme-linked immunoassay (ELISA), based on the principal of competitive binding. The assay measures both Vitamin D2 and D3. The total assay procedure run time is 2.5 hours.
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Material not included:	1. Precision pipettes 2. Disposable pipette tips 3. ELISA reader capable of reading absorbance at 450 nm 4. Flat-head Vortex mixer 5. Plate shaker 6. Graph paper

Target Details

Target:	25-OH Vitamin D

Target Details

Alternative Name:	25-hydroxy Vitamin D (25-OH Vitamin D Products)
Background:	Vitamin D is a steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. Vitamin D has two isomers: Vitamin D2 and Vitamin D3. Vitamin D2 is obtained from dairy products whereas Vitamin D3 is produced in the skin after exposure to ultraviolet light. In the liver, Vitamin D is hydroxylated at its carbon 25 to form 25-OH Vitamin D. This metabolite is the predominant circulating form of Vitamin D and is considered to be an accurate indicator of the general Vitamin D status of an individual. Vitamin D deficiency has been linked to many diseases including osteoporosis, rickets, osteomalacia, cancers, and cardiovascular 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 \\

Application Details

Application Notes:	We recommend that each laboratory uses 25-OH Vitamin D controls to validate the performance of reagents.
Sample Volume:	10 µL
Assay Time:	2.5 h
Plate:	Pre-coated
Reagent Preparation:	Before running the test, prepare the following: 1. Standards and Reagents: Standards are serum-based solutions and stable when stored at -2-8°C, protected from light, until the expiration date on the label. Equilibrate the needed volume of standards and reagents to room temperature before use. 2. 51X Biotin conjugate: Immediately before use, prepare 1X working solution at 1:51 with assay diluent (e.g. Add 0.1ml of the 50x Vitamin D-Biotin conjugate concentrate to 5ml of assay diluent). Remaining Assay Diluent must be stored at 2-8°C in dark and tightly capped. 3. Prepare 1X Wash Buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-24 °C).
Assay Procedure:	• anti-Vitamin D antibody coated wells are incubated with Vitamin D standards, controls, samples, and Vitamin D-Biotin conjugate at room temperature for 90 minutes. • During the incubation, a fixed amount of biotin-labeled vitamin D competes with the endogenous Vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti Vitamin D antibody. • Following a wash step, bound Vitamin D-Biotin is detected with Streptavidin-HRP.

Application Details

	Streptavidin-HRP conjugate immunologically bound to the well progressively decreases as the concentration of Vitamin D in the specimen increases. Unbound SA-HRP conjugate is then removed and the wells are washed. • Next, a solution of TMB Reagent is added and incubated at room temperature for 30 minutes, resulting in the development of blue color. • The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. • A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The color intensity will be inversely proportional the amount of 25(OH)D in the sample.
Calculation of Results:	Results are expressed in ng/mL. Note: To convert to nmol/L, multiply results by 2.5. Example: 10 ng/mL = 25 nmol/L.
Restrictions:	For Research Use only

Handling

Precaution of Use:	1. Potential biohazardous materials: The standards contain human source components which have been tested and found nonreactive 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 virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, Biosafety in Microbiological and Biomedical Laboratories. 1984. 2. This kit is intended for Research Use Only. Not for use in diagnostic procedures. 3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. 4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. 5. It is recommended that standards, control and serum samples be run in duplicate 6. 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.
Handling Advice:	Avoid repeated freeze-thaw cycles. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use, samples must be mixed before analysis. Serum, heparinized plasma or EDTA plasma samples can be used for the assay. - For serum, collect whole blood by venipuncture and allow clotting. - For plasma, mix the sample by gentle

Handling

	inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. Do not use hemolyzed samples.
Storage:	4 °C
Storage Comment:	The specimens may be refrigerated at 2-8 °C for two weeks. For long term storage, they can be stored at -20 °C.

Publications

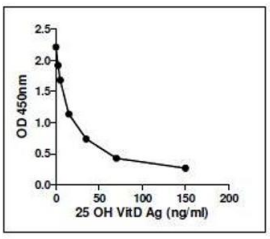
Product cited in:

MacMillan, Lamberti, Moulton, Geilich, Webster: "Similar healthy osteoclast and osteoblast activity on nanocrystalline hydroxyapatite and nanoparticles of tri-calcium phosphate compared to natural bone." in: **International journal of nanomedicine**, Vol. 9, pp. 5627-37, (2014) (PubMed).

Ganzetti, Campanati, Scocco, Brugia, Tocchini, Liberati, Giuliodori, Brisigotti, Offidani: "The potential effect of the tumour necrosis factor-? inhibitors on vitamin D status in psoriatic patients." in: **Acta dermato-venereologica**, Vol. 94, Issue 6, pp. 715-7, (2014) (PubMed).

Ham, Longhi, Lahiff, Cheifetz, Robson, Moss: "Vitamin D levels in adults with Crohn's disease are responsive to disease activity and treatment." in: **Inflammatory bowel diseases**, Vol. 20, Issue 5, pp. 856-60, (2014) (PubMed).

Images



25(OH)D, (ng/ml)	Absorbance (450nm)
0	2.214
1.25	1.920
2.5	1.683
10	1.137
35	0.737
70	0.425
150	0.267

ELISA

Image 1. Seven standard levels are included for each run. A typical standard curve is shown below.