Vitamin D total

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25-Hydroxyvitamin D Total (ELISA)

REF: DS167701 Σ 96

Intended use
The DiaSino Vitamin D total assay is an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of total 25-hydroxy Vitamin D (Vitamin D total) in human serum. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of vitamin D sufficiency. For professional use only.

Summary
Vitamin D is a fat-soluble steroid hormone precursor that is mainly produced in the skin by exposure to sunlight. Vitamin D is biologically inert and must undergo hydroxylation steps to become active.[1] Our body can only synthesize vitamin D3. Vitamin D2 is taken up with fortified food or given by supplements. Physiologically, vitamin D3 and D2 are bound to the vitamin D-binding protein (VDBP) in plasma and transported to the liver to become 25-hydroxyvitamin D (25-OH Vitamin D). As vitamin D (25-OH) represents the major storage form, its blood concentration is used to assess the overall vitamin D status. More than 95% of vitamin D (25-OH), measurable in serum, is vitamin D3 (25-OH) whereas vitamin D2 (25-OH) reaches measurable levels only in patients taking vitamin D2 supplements.[1,2,3]
Vitamin D is essential for bone health. In children, severe deficiency leads to rickets. In elderly, the risk of falling has been attributed to vitamin D deficiency due to muscle weakness. Moreover, low vitamin D (25-OH) concentrations are associated with lower bone mineral density. Insufficiency has also been linked to diabetes, cardiovascular disease, and autoimmune diseases.[1]

Test principle
Competitive principle**.** Total duration of assay: **85 minutes.**
- Sample, 25-Hydroxyvitamin D derivant coated microwells and enzyme labeled Anti- 25-Hydroxyvitamin D are combined.
- During the incubation, 25-Hydroxyvitamin D derivant coated on microwells and 25-Hydroxyvitamin D present in the sample compete for binding to the enzyme labeled antibodies.
- After washing, a complex is generated between the solid phase and enzymelinked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The color intensity is inversely proportional to the amount of 25-Hydroxyvitamin D in the sample.

Reagents
Materials provided
- **Vitamin D total Coated Microplate - - symbol** VIT D PLATE 8 x 12 strips, 96 wells, pre-coated with 25-Hydroxyvitamin D derivant.
- **Vitamin D total Calibrators - symbols** VIT D CAL A-F 6 vials, 1 mL of each, ready to use; Concentrations: 0(A), 7.5(B), 15(C), 30(D), 75(E) and 150(F) ng/ml.
- **Vitamin D total Incubation Buffer - symbol** VIT D BUFFER 1 vial, 6.0 mL. Contains 0.2% ProClin300 preservative.
- **Vitamin D total Enzyme Conjugate - symbol** VIT D CONJ Ready to use, 1 vial, 6.0 mL of HRP (horseradish peroxidase) labeled sheep monoclonal Anti-25-Hydroxyvitamin D in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.2% ProClin300 preservative.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 1 piece.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.2% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Incident report
Any suspected serious incidents related to this assay shall be immediately reported to DiaSino, DiaSino's Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.

Storage and stability
- Store at 2-8°C.
- Seal and return unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum is recommended for this assay.
- Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8°C, and 1 month at -20°C. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Calibration
The DiaSino Vitamin D assay has been standardized against the Elecsys Vitamin D total II, which are traceable to the ID-LC-MS/MS 25-hydroxyvitamin D Reference Measurement Procedure.[4,5]
Recalibration is recommended when a new reagent lot is used, or the quality controls are out of specified range.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Note: Ensure the patients' samples, reagents (Conjugate, Incubation Buffer, Calibrators) are at ambient temperature (18-25 °C) at least 30 minutes before measurement. Mix all reagents through gently inverting prior to use.
Incubation Buffer must be mixed thoroughly otherwise the testing results will get influenced.
- Use only the number of wells required and format the microplates wells for each calibrator and sample to be assayed.
- Add **50 µL** of **Calibrators** or **Samples** to each well.
- Add **50 µL** of **Incubation Buffer** to each well.
- **Shake** the **microplate** gently for **5 minutes** to mix.
- Add **50 µL** of **Enzyme Conjugate** to each well.
- Shake the microplate gently for 30 seconds to mix.
- Cover the plate with a plate lid and incubate at **37 °C** for **60 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µL** of **Wash Solution,** decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
- Add **100 µL** of **Substrate** to each well.
- Cover and incubate at **ambient temperature** (18-25°C) in the dark for reaction for **20 minutes**. Do not shake the plate after substate addition.
- Add **50 µL** of **Stop Solution** to each well.
- Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.

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- Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader.
- The results should be read within 30 minutes of adding the stop solution.

Calculation
- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in ng/mL for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. **Point- to-Point** method is suggested to generate a calibration curve.

The following data and calibration curve is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (ng/mL)	Absorbance
Calibrator A	0	2.818
Calibrator B	7.5	2.060
Calibrator C	15	1.522
Calibrator D	30	1.063
Calibrator E	75	0.576
Calibrator F	150	0.284
Control 1	16.41	1.479
Control 2	37.48	0.982
Sample	21.41	1.326

Limitations - interference
- The assay is unaffected by icterus (bilirubin < 600 µmol/L or < 35 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.9 g/dL), lipemia (Intralipid < 1200 mg/dL), and biotin < 94 nmol/L or < 23 ng/mL.
- Criterion: Recovery within ± 10 % of initial value.
- Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
2.0-150 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 2.0 ng/mL. Values above the measuring range are reported as > 150 ng/mL.

Lower detection limit
2.0 ng/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1+2 SD, repeatability study, n=21).

Expected values

Level	ng/mL
Deficient	<10
Insufficient	10-29
Sufficient	30-100
Potential Toxicity	>100

Measurement with the DiaSino 25-OH Vitamin D assay on 172 healthy serum samples from test subjects in China yielded the above values (2.5th-97.5th percentile): the average level is around 20 ng/mL.

Specific performance data
Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using DiaSino reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

		Repeatability		Intermediate precision	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human Serum 1	8.2	0.6096	7.4	0.6972	8.5
Human Serum 2	21.4	1.3268	6.2	1.5423	7.2
Human Serum 3	33.5	2.0544	6.1	1.9754	5.9
PC Universal 1	13.3	0.9773	7.3	0.9877	7.4
PC Universal 2	30.1	1.4050	4.7	1.8523	6.2

Method comparison
A comparison of the DiaSino 25-OH Vitamin D assay (y) with Elecsys Vitamin D II total (x) using 105 clinical samples gave the following correlations:

Linear regression
y = 0.9437x + 0.8223
r = 0.9594
The sample concentrations were between approx. 2.0 and 148 ng/mL.

Analytical specificity
A study was performed based on guidance from CLSI EP07-A2 to evaluate the cross-reactivity of the assay with other vitamin D metabolites. Samples containing the cross-reactants were prepared at three 25-hydroxyvitamin D concentrations (25, 40 and 60 ng/mL). The % cross-reactivity was calculated for each sample using the equation below and normalized to the cross-reactivity of 25-hydroxyvitamin D3.[6]

$$\%\ \text{cross-reactivity} = \frac{\text{mean conc. of spiked sample - mean conc. of unspiked sample}}{\text{spiked concentration}} \times 100\%$$

The mean results from this study are summarized in the following table:

Cross-reactant	Concentration added, ng/mL	Mean cross-reactivity %
25-hydroxyvitamin D_3	50	99.80
25-hydroxyvitamin D_2	50	97.50
3-epi-25-hydroxyvitamin D_3	50	113.10
3-epi-25-hydroxyvitamin D_2	50	92.20
1,25-dihydroxyvitamin D_3	100	n.d
1,25-dihydroxyvitamin D2	100	n.d
Vitamin D_3	1000	0.85
Vitamin D_2	1000	0.41

Functional sensitivity
2.10 ng/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

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2. Houghton, L.A., Vieth, R. (2006). The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr, 84:694-697.
3. Hart, G.R., Furniss, J.L., Laurie, D., et al. (2006). Measurement of vitamin D Status: background, clinical use and methodologies. Clin Lab, 52(7-8):335-343
4. Sempos CT, Vesper HW, Phinney KW, et al. The Vitamin D Standardization Program (VDSP). Vitamin D Status as an International Issue: National Surveys and the Problem of Standardization. Scand J Clin Lab Invest 2012;72(Suppl 243):32-40.
5. ThienpontLM,StepmanHCM,VesperHW.Standardizationof Measurements of 25-Hydroxyvitamin D3 and D2. Scandinavian Journal of Clinical & Laboratory Investigation, 2012;72(Suppl 243):41-49.
6. Carter GD, Jones JC, Berry JL. The anomalous behaviour of exogenous 25-hydroxyvitamin D in competitive binding assays. J Steroid Biochem 2007;103(3-5): 480-482.

Symbols

IVD In vitro diagnostic medical device	8°C / 2°C Temperature limit	Consult instructions for use	REF Catalog number
LOT Batch code	Date of manufacture	Use-by date	Σ Contains sufficient for <n> tests
Manufacturer	Do not use if package is damaged and consult instructions for use	European Conformity	EC REP Authorized representative in the European Community

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