Vitamin D

infinosis™

25-Hydroxy Vitamin D total (FIA)

REF: IN037701 Σ 25

Intended use
The infinosis™ Vitamin D is a fluorescence immunoassay for the in vitro quantitative determination of total 25-hydroxy Vitamin D in Human serum or plasma. For professional use only.

Summary
References[1-4]
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. 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.
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.

Test principle
Sandwich principle. Total duration of assay: **15 minutes**
Sample is added to the sample well of the test, then the fluorescence-labeled detector anti-25-OH Vitamin D antibody binds to 25-OH Vitamin D antigen in blood specimen. As the sample mixture migrates on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and 25-OH Vitamin D are captured to anti-TSH antibody that has been immobilized on test strip. More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by instrument for infinosis™ tests to show 25-OH Vitamin D concentration in the sample.

Reagents
Materials provided
- **Test Cartridge**, 25 pcs, individually packaged
- **ID Chip** or **QR code of Calibration Curve**, 1 pcs
- **Sample Buffer,** 25 tubes
- **IFU**, 1 copy

Materials required (but not provided)
- 25-OH Vitamin D control (DiaSino control is recommended)
- Infinosis™ FIA analyzer
- Transfer pipette set (100 μL size)
- Centrifuge (for plasma and serum only)
- Timer

Precautions and warnings
- For in vitro diagnostic use only.
- Carefully follow the instructions and procedures described in this instructions before testing.
- The Test cartridge should remain in its original sealed pouch until ready to use. Do not use it if the pouch is damaged or the seal is broken.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different Lots.
- Don't use Test cartridge if its Lot does not match with ID Chip that is inserted onto the instrument.
- The Infinosis™ 25-OH Vitamin D assay should be used only in conjunction with the instrument for infinosis™ tests.
- The tests should be applied by professionally trained staff working in certified laboratories at some remove from the patient and clinic at which the sample is taken by qualified medical personnel.
- infinosis™ 25-OH Vitamin D assay is single use only. Do not re-use it.
- The Test cartridge and Analyzer should be used away from vibration and magnetic field. During normal usage, the Test cartridge may introduce minute vibration, which should be regarded as normal.
- Use separate clean pipette tips and detector buffer vials for different specimens. The pipette tips and detector buffer vials should be used for one specimen only.
- Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.
- Blood specimens, used Test cartridges, pipette tips and detector buffer vials are potentially infectious. Proper laboratory safety techniques, handing and disposal methods should be followed in accordance with standard procedures and relevant regulations observed by microbiological hazard materials.
- The results should be interpreted by the physician along with clinical findings and other laboratory test results.

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 all the test kit at 2-30°C, the stability is up to the expiration date printed on package.
- Test cartridge and sample buffer should be used within 1 hour after opening the pack.

Specimen collection and preparation
- The test can be performed with either serum or plasma.
- Collect serum samples in accordance with correct medical practices.
- Using standard phlebotomy procedure, collect a venipuncture whole blood specimen using a blood collection tube. If collecting plasma use a blood collection tube containing suitable anticoagulant (EDTA recommended).
- Separate the serum/plasma from blood as soon as possible to avoid hemolysis.
- Test should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long-term storage, specimens should be kept below -20°C.

Quality control
- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with infinosis™ tests. For more information regarding obtaining the control materials, contact DiaSino Laboratories Co., Ltd for assistance.

Test setup
- Ensure that the lot number of the cartridge matches that of the sample buffer, and the ID Chip.
- If the sealed cartridge and sample buffer have been stored in refrigerator, place them at room temperature (18-25 °C) at least 30 minutes before measurement.
- Turn on the instrument for infinosis™ tests.
- Refer to the *'instrument for infinosis™ tests Operation Manual'* for the complete information and operating instructions.

Test procedure
1. Insert ID Chip into the instrument for infinosis™ tests or Scan the QR code to read the calibration curve.
2. Using a pipette to transfer **20 μL** of sample (Human plasma/serum) to the **sample buffer tube** provided in the kit.
3. Close the lid of the sample mixing tube and mix the sample thoroughly for **5-10 seconds** by tapping or inverting the tube.
4. Pipette out **100 μL** of **sample mixture** and load it onto the sample well on the cartridge.
5. Leave the sample-loaded cartridge at room temperature for **15 minutes.**
6. Insert the sample-loaded cartridge into the cartridge holder of instrument for infinosis™ tests.
   Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder.
7. Press **"Test"** button on the instrument for infinosis™ tests.
8. Instrument for infinosis™ tests will start scanning the sample-loaded cartridge immediately.

Limitations - interference

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- The assay is unaffected by icterus (bilirubin < 600 □ mol/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.

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 infinosis™ 25-OH Vitamin D assay on 172 healthy serum samples from test subjects in China yielded the above values (2.5^{th}-97.5^{th} percentile): the average level is around 20 ng/mL.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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

Precision
Intra-assay
Determined by using 10 tests in the same batch to test with 25-OH Vitamin D control, CV ≤ 15%

Inter-assay
Determined by using 3 tests in 3 random and continuous batches to test with 25-OH Vitamin D control, CV ≤ 20%

Method comparison
A comparison of the infinosis™ 25-OH Vitamin D assay (y) with the Roche Elecsys 25-OH Vitamin D total II (x) using clinical samples gave the following correlation:
Number of samples measured: 312
Linear regression

y = 0.9437x + 0.8223

r = 0.9594

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.[4]

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

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

References

1. Holick, M.F. (2007). Vitamin D de ciency. N Engl J Med, 357:266-281.
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. 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	2°C 30°C Temperature limit	Consult instructions for use	REF Catalog number
LOT Batch code	Date of manufacture	Use-by date	Contains sufficient for <n> tests
Manufacturer	Do not re-use	Do not use if package is damaged and consult instructions for use	CE European Conformity
EC REP Authorized representative in the European Community			

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