ISE S.r.l. Vitamin D (100 test) – Instructions for use

IVD

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R1: 1x 15,5 mL R2: 1x 2,8 mL **R0000000005**

REF

Intended Use
The Vitamin D assay is intended for use in clinical laboratories for the quantitative determination of 25-OH vitamin D /vitamin D) in human serumand plasma, using automated chemistry analyzers. Measurement of vitamin D is used for the assessment of the vitamin D sufficiency. ***For in Vitro Diagnostic use only.***

Clinical significance
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 forms: 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, and osteomalacial. 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 "Total 25-OH Vitamin D". Accurate monitoring of total 25-OH Vitamin D level is critical in clinical setting.

Assay Principle
The Vitamin D assay is a direct particle-enchained immune-turbidimetric assay. The assay's proprietary reagents are designed to dissociate vitamin D from vitamin D binding proteins, found in serum or plasma specimens, while particles coated with anti-Vitamin D antibodies bind to the dissociated vitamin D, thereby causing agglutination. This agglutination is detected as an absorbance change (700 nm), with the magnitude of the change being proportional to the quantity of total vitamin D in the sample. Specimen concentrations are determined by interpolation from a 5-point calibration curve prepared from calibrators of known concentrations

Kit R1: 1 x 15,5 mL R2: 1 x 2,8mL code R0000000005
Reagent 1: no. 1 Vials 15,5 mL Ready to Use
Reagent 2: no. 1 vials 2,8 mL Ready to Use

Reagents Provided

Reagent 1:
Phosphate buffer solution (<100 mM), 0,1 % sodium azide
Reagent 2:
Suspension of latex particles (0,5%) coated with anti-vitamin D antibodies, ready to use

Reagents required but not supplied
1. Calibrators and Controls*

Key Reference	**Description**
R0000000006	Vitamin D calibrator
R0000000007	Vitamin D control

Materials required but not provided
An analyser capable of dispensing two reagents and measuring absorbance at around 700 nm with temperature control (37°C).

Reagent handling
The Vitamin D assay reagents are liquid stable, ready to use reagents. Mix by inverting at least 10 times before use.

Reagent stability and storage
The reagents are stable when stored at 2-8°C until the expiration date on the label. Do not mix reagents of different lots. **DO NOT FREEZE.**

Sample collection and handling
Serum, K2-EDTA plasma, K3-EDTA plasma or Li-heparin plasma samples can be used for the assay. Method compharison of K2-EDTA plasma samples versus samples yielded a regression equation of u=1.0198x-0.4985 and R2=0.996. method comparions of K3-EDTA plasma samples versus serum samples yielded a regression equation of y= 1.0378x-1.2959 and R2=0.9944. Method comparison of Li-heparin plasma samples versus serum samples yielded a regression equation of y= 1.0475x-1.3749 and R2=0.9947. For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. The specimens may be refrigerated at 2-8°C for up to one week. For long term storage, they can be stored at -20°C or below. Avoid repeated freeze-thaw cycles (up to three cycles are acceptable). Do not use highly turbid or highly hemolyzed serum or plasma samples. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed well before analysis.

Precautions
1. For *in vitro* diagnostic use only. Rx Only.
2. As with any laboratory test, results should be interpreted considering all other test results and the status of the sample. Do not use the reagent, calibrator and control after the expiration date labeled on the outer box.
3. Assay calibration frequency is depending on instrument used. Additionally the assay should be recalibrated and controls run with each new lot of reagents.
4. Avoid ingestion and contact with skin and eyes. See safety data sheet.
5. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures.
6. Calibrators and controls contain human source material. Each donor unit of serum in the preparation of these materials were tested by approved methods and found negative Heptitis B surface antigen (HBsAg), and Hepatitis C Virus antibody (HCV). Because no method can offer complete assurance as to the absence of infectious agents, this material and all samples should be handled as though capable of transmitting infectious disease and such biohazardous material should be disposed of according to relevant local or state regulations.
7. Additional safety information concerning storage and handling of this product is provided within the MSDS for this product.

Warnings
The reagent, calibrators and controls contains < 0.1 % sodium azide, NaN3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal flush with a large volume of water to prevent sodium azide buildup.

Assay procedure
Below is a general example of assay test scheme and the specific application parameters for the Beckman AU 680 analyzer.

R1 160 µL
Sample 3 µL
Incubated at 37 °C for 4 minutes

I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 0774579365; FAX +39 0774579305
www.logotech-ise.com E-mail: info@logotech-ise.com

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Add R2 40 µL
While 4 minutes read the reaction at 700 nm

Calibration
5 level of the Vitamin D calibrator cod. R0000000006 are provided separately and ready to use.

Quality control
We recommend that each laboratory use Vitamin D controls to validate the performance of the Vitamin D assay reagents. A set of normal and abnormal ranges of Vitamin D controls are available cod. R0000000007 Vitamin D control high and cod. R0000000008 Vitamin D control low. The range of acceptable control limits should be established by individual laboratories.

Results
Results are expressed in ng/mL. Note: Samples with values greater than 147.8 ng/mL should be reported as > 147.8 ng/mL. Samples with values less than 7.6 ng/mL should be reported as < 7.6 ng/mL.

Reference range
Following C28-A3 approved guideline third edition, reference range of the Vitamin D assay was determined by measuring the 25-OH vitamin D serum concentrations of a USA population of 145 apparently healthy adults, 21-67 years old, during three different geographical locations: 47 from Pennsylvania (Northern USA), 49 from Tennessee (Central USA) and 49 from Texas (Southern USA). All 145 individuals did not have kidney disease, G1 disease, liver disease, calcium – levels related disease, thyroid disease, parathyroid disease, seizures, chronic disease or bariatric surgery. The central 95 % of population was found to have a 25-OH vitamin D concentrations ranging between 7.2 and 41.6 ng/mL, with a mean concentration of 20.1 ng/ml.

Limitations

1. The assay is designed for use with human serum and plasma samples only.
2. As with any diagnostic test it is possible that technical, procedural errors as well as substances and factors not listed may interfere with the proper functioning of the test kit.
3. Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immuunoassays. Patients routinely exposed to animals, animal serum products or other immunogenic products that may elicit heterophilic antibody production against the assay's reagents can be prone to this interference and anomalous values may be obtained. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions in an adult population.

Performance characteristics

The following performance data was obtained on the Beckman AU680 chemistry analyser.

Sensitivity
The LOB, LOD and LOQ of the assay were determined following CLSI EP17-A2 guideline.

Limit of Blank LoB
Vitamin D – depleted serum was assayed with the Vitamin D assay in five independent (over 5 days) runs with 12 replicates per run. The LoB was calculated as the mean of the 57th and 58th highest values for the blanks. The LoB of the assay ws 1.2 ng/mL.

Limit of Detection LoD
Five very low vitamin D serum samples were measured in four independent runs (over 4 days), with 3 replicates per run. The LoD was defines as LoD =LoB + (1.645 * Standard Deviation of Low samples). The LoD of the assay was 2.9 ng/mL.

Limit of Quantitation LoQ
Five low Vitamin D samples were measured in 40 replicates obtained from five independent runs (5 days). The LoQ was measured as the lowest concentration with a CV of 20 %. The claimed LoQ of the assay ws 7.6 ng/mL.

Method comparison
The method comparison of the assay was evaluated following CLSI EP9-A3 guideline. A total of 171 serum samples were tested in comparison with a legally marketed 25-OH Vitamin D enzyme immunoassay. The results for 171 serum samples are shown in the below table:

Deming Regression Analysis	95 % Confidence Interval
Slope	1062 (1028 to 1095)
Intercent	-3.03 (-4.94 to – 1.11)
Correlation coefficient	0.9785 (0.970 to 0.9841)
Predicate Range (ng/mL)	8.4 to 146.8

Precision
Precision was evaluated according to the CLSI EP5-A2 guideline. Controls and samples were measured daily over the span of 20 days. Using three lots of reagents and one chemistry analyser. 40 independent runs were performed on each specimen. Each run produced two measurements. 80 data points were obtained per specimen. Results are shown below:

25-OH Vitamin D ng/mL			Within-run		Between run		Total	
Specimen	N	Mean	SD	%CV	SD	%CV	SD	%CV
Control #1	80	21.7	0.9	3.9%	0.6	2.8%	1.3	6.2%
Control #2	80	42.5	1.0	2.4%	0.8	2.0%	1.7	3.9%
Control #1	80	11.1	0.9	8.3%	0.5	4.4%	1.8	16.6%
Control #2	80	18.2	0.9	4.9%	0.7	3.9%	1.6	8.7%
Control #3	80	22.1	0.8	3.8%	0.8	3.8%	1.2	5.6%
Control #4	80	42.8	0.9	2.0%	1.0	2.1%	1.3	3.1%
Control #5	80	59.5	1.0	1.7%	0.7	1.2%	1.6	2.7%
Control #6	80	80.2	1.3	1.6%	1.1	1.4%	2.0	2.5%
Control #7	80	99.5	1.8	1.8%	1.5	1.6%	2.7	2.8%
Control #8	80	117.6	2.2	1.9%	2.0	1.7%	3.7	3.2%
Control #9	80	139.2	2.7	1.9%	2.6	1.8%	4.1	2.9%

Linearity
Eleven levels of linearity were prepared by diluting a high serum sample with vitamin D – depleted serum. Linearity levels were prepared according to the CLSI EP6-A guideline. Measurements

were done in triplicates. The assay was found to be linear between 7.6 and 147.8 ng/mL.

Interference

Interference studies were conducted according to the CLSI EP7-A2 guideline. The acceptance criterion was set at 10 % or less deviation between the spiked sample and the control. The assay's result were not significantly affected by the following endogenous substances:

Substance	Tolerance	Unit
Free bilirubin	40	mg/dL
Conjugated bilirubin	40	mg/dL
Hemoglobin	600	mg/dL
Total protein	12.0	g/dL
Triglycerides	1000	mg/dL
Rheumatoid factor (RF)	200	IU/mL

The assay's results were also not significantly affected by the following exogenous substances:

Substances	Tolerance	Unit
Acetaminophen	20.0	mg/dL
Acetyl salicylic acid	60.0	mg/dL
Ampiellin	5.3	mg/dL
Ascorbate	3.0	mg/dL
Biotin	100.0	mg/dL
Carbamazepine	3.0	mg/dL
Cefotaxime	180.0	mg/dL
Chloramphenicol	5.0	mg/dL
Creatinine	30.0	mg/dL
Digoxin	6.1	mg/dL
Ethanol	400.0	mg/dL
Ethosuximide	25.0	mg/dL
Furosemide	6.0	mg/dL
HAMA	350	ng/mL
Heparin	3.0	U/mL
Ibuprofen	50.0	mg/dL
Lidocaine	1.2	mg/dL
Lithium acetate	2.2	mg/dL
Noradrenalin	4.0	Ug/mL
Rifampicin	5.0	mg/dL
Theophylline	4.0	mg/dL
Urea	300.0	mg/dL
Uric acid	20.0	mg/dL
Valrpoid acid	50.0	mg/dL
Vancomycin	10.0	mg/dL

Cross – reactivity of the Vitamin D assay was determined by adding various vitamin D metabolites to serum pool samples. Based on the results in the table below, the assay did not cross-react with vitamin D2 or vitamin D3. The assay recovered 25-OH vitamin D2 and 25-OH vitamin D3 similarly. Cross-reactivity results are summarized in the table below:

Compound	Concentration tested	Cross-reactivity *
25-OH Vitamin D3	100.0 ng/mL	100 %
25-OH Vitamin D2	100.0 ng/mL	106.9 %
Vitamin D3	100.0 ng/mL	-0.8%
Vitamin D2	100.0 ng/mL	-1.7 %
1.25 –(OH)2 Vitamin D3	580.0 pg/mL	0.2 %
1.25 –(OH)2 Vitamin D2	580.0 pg/mL	-0.5 %
24R,25-(OH)2 Vitamin D3	100.0 ng/mL	118.8%
3-epi-25-OH Vitamin D3	100.0 ng/mL	31.0 %
3-epi-25-OH Vitamin D2	100.0 ng/mL	36.5 %

*% Cross reactivity = (corrected assay value / concentrations spiked)*100

Paricalcitol (Zemplar ®) did not significantly cross-react with the assay when present at 5 ng/mL.

Disposal of reagent

Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

The product is in conformity with D.L: 8 September 2000, no. 332 "Actuation of the directive 98/79/EC regarding in vitro medical diagnostic devices".

Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 0774579365; FAX +39 0774579305
www.logotech-ise.com E-mail: info@logotech-ise.com

 = Temperature limitation

 = Instruction for use

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I.S.E S.r.l.
Via Delle Driadi, 45 – 00133 Roma
Tel.+39 0774579365; FAX +39 0774579305
www.logotech-ise.com E-mail: info@logotech-ise.com