25-Hydroxy Vitamin D Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL096VD	1 x 40 mL	REAGENT 1	2-8°C
	1 x 10 mL	REAGENT 2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of total 25-OH Vitamin D in human serum and plasma on automated and semi-automated analysers.

SUMMARY AND EXPLANATION:

Measurement of 25-hydroxy Vitamin D (25-OH Vitamin D) is for the assessment of vitamin D sufficiency. 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 osteomalacia[1]. 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 settings[1-5].

PRINCIPLE OF THE TEST:

The Glenbio Vitamin D Assay is a direct particle-enhanced immuno-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.

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
This product is not hazardous under EU specifications. Material Safety Data Sheet is available upon request.
The reagents contain sodium azide (<0.1%) as a preservative. Do not ingest. Avoid contact with skin and eyes.

Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

COMPONENT COMPOSITION:

Reagent 1	Phosphate buffer solution (< 100mM), 0.1% sodium azide.
Reagent 2	Suspension of latex particles(<0.5%) coated with anti-vitamin D antibodies.
Calibrators	Human serum containing specific amounts of 25-OH Vitamin D and 0.1% sodium azide.

REAGENT PREPARATION AND STABILITY:

Reagents are ready to use.
Before use, mix reagent by gently inverting each bottle. If stored and handled properly, component is stable until expiry date stated on the label. Once opened the reagent should be kept capped in their corresponding reagent bottles. Do not mix reagents with different lot numbers.

TYPE OF SPECIMEN: [5]

Serum, K_3-EDTA plasma or Li-heparin plasma samples can be used for the assay. Method comparison of K_2-EDTA plasma samples versus serum samples yielded a regression equation of y = 1.0198x - 0.4985 and an R^2 = 0.996. Method comparison of K_3-EDTA plasma samples versus serum samples yielded a regression equation of y = 1.0378x – 1.2959 and an R^2 = 0.9944. Method comparison of Li-heparin plasma samples versus serum samples yielded a regression equation of y = 1.0475x – 1.3749 and R^2 = 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 one week. For long term storage, they can be stored at -20°C. Avoid repeated freeze-thaw cycles. Do not use highly turbid or highly haemolysed 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.

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalogue No.	Description	Catalogue No.
Vitamin D Calibrator Set	GL955	General Laboratory Equipment	N/A
Vitamin D Control Set	GL956	Photometer	N/A

Assay procedure:
Refer to relevant user's manual for instructions on instrument start-up, loading components and samples, calibration, sample testing procedures, calculating and reporting results.

Calibration:
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the analyser in use.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior to reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Laboratory QC Programme.

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 and samples with values less than 7.6 ng/mL should be reported as <7.6 ng/mL.

REFERENCE RANGES:

The reference range of this 25-OH Vitamin D assay was determined by measuring the 25-OH Vitamin D serum concentrations of a population of 145 apparently healthy adults. The central 95% of the population was found to have 25-OH vitamin D concentrations ranging between 7.2 to 41.6 ng/mL. The median concentration was 20.1 ng/mL.

Each laboratory should establish its own reference range and results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
Linear between 7.6 and 147.8 ng/mL.

Sensitivity:
Limit of Blank (LoB) – 1.2 ng/mL.
Limit of Detection (LoD) – 2.9 ng/mL.
Limit of Quantitation (LoQ) – 7.6 ng/mL.

Precision:

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%
Sample #1	80	11.1	0.9	8.3%	0.5	4.4%	1.8	16.6%
Sample #2	80	18.2	0.9	4.9%	0.7	3.9%	1.6	8.7%
Sample #3	80	22.1	0.8	3.8%	0.8	3.8%	1.2	5.6%
Sample #4	80	42.8	0.9	2.0%	1.0	2.4%	1.3	3.1%
Sample #5	80	59.5	1.0	1.7%	0.7	1.2%	1.6	2.7%
Sample #6	80	80.2	1.3	1.6%	1.1	1.4%	2.0	2.5%
Sample #7	80	99.5	1.8	1.8%	1.5	1.6%	2.7	2.8%
Sample #8	80	117.6	2.2	1.9%	2.0	1.7%	3.7	3.2%
Sample #9	80	139.2	2.7	1.9%	2.6	1.8%	4.1	2.9%

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

Substance	Concentration	Substance	Concentration
Conjugated Bilirubin	40 mg/dL	Free Bilirubin	40 mg/dL
Haemoglobin	600 mg/dL	Triglycerides	1000 mg/dL
Rheuamtoid Factor (RF)	200 IU/mL	Total protein	12.0 g/dL

* Acceptance criteria was set at 10% or less deviation.

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

Substance	Tolerance	Unit	Substance	Tolerance	Unit
Acetaminophen	20.0	mg/dL	HAMA	350	ng/mL
Acetyl Salicylic Acid	60.0	mg/dL	Heparin	3.0	U/mL
Ampicillin	5.3	mg/dL	Ibuprofen	50.0	mg/dL
Ascorbate	3.0	mg/dL	Lidocaine	1.2	mg/dL
Biotin	100.0	ng/mL	Lithium Acetate	2.2	mg/dL
Carbamazepine	3.0	mg/dL	Noradrenalin	4.0	ug/mL
Cefotaxime	180.0	mg/dL	Rifampicin	5.0	mg/dL
Chloramphenicol	5.0	mg/dL	Theophylline	4.0	mg/dL
Creatinine	30.0	mg/dL	Urea	300.0	mg/dL
Digoxin	6.1	ng/mL	Uric Acid	20.0	mg/dL
Ethanol	400.0	mg/dL	Valproid Acid	50.0	mg/dL
Ethosuximide	25.0	mg/dL	Vancomycin	10.0	mg/dL
Furosemide	6.0	mg/dL			

Cross-reactivity of this 25-OH Vitamin D Assay was determined by adding Vitamin D metabolites to serum pool samples. Based on the results in the table below, the assay did not cross react with Vitamin D2 and Vitamin D3 and the assay recovers both 25-OH Vitamin D2 and 25-OH Vitamin D3 similarly. Cross-reactivity with various Vitamin D metabolites is 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	33.0%
3-epi-25-OH Vitamin D2	100.0 ng/mL	36.5%

**% Cross-reactivity = (Corrected Assay Value /Concentration Spiked)*100*

Method Comparison:
Using 171 samples, a comparison, between this test (y) and another commercially available test (x), gave the following results:

Deming Regression Analysis	95% Confidence Interval
Slope	1.062 (1.028 to 1.095)
Intercept	-3.03 (-4.94 to -1.11)
Correlation Coefficient	0.9785 (0.970 to 0.9841)
Range	8.4 - 146.8

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SYMBOLS:

The following symbols are used in the labelling of Glenbio Ltd. systems:

IVD — In Vitro Diagnostics
REF — Catalogue No
LOT — Batch Code
CONT — Content
REAG — Reagent
CE — CE Mark - Device comply with the Directives 98/79/EC
Storage temperature
Consult Instruction for Use
Expiry Date (Last day of the month)
Manufactured By

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