Snibe Diagnostic

CE

REF 130211004M: 100 tests
130611004M: 50 tests

MAGLUMI® 25-OH Vitamin D (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of 25-OH Vitamin D in human serum using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

25-hydroxyvitamin D, also known as Calcifediol, calcidiol, 25-hydroxycholecalciferol, or (abbreviated 25-OH Vitamin D), is a prehormone that is produced in the liver by hydroxylation of vitamin D3 (cholecalciferol) by the enzyme cholecalciferol 25-hydroxylase which was isolated by Michael F. Holick[1]. 25-hydroxy vitamin D is then converted in the kidneys (by the enzyme 25(OH) D-1α-hydroxylase) into calcitriol (1, 25-(OH) 2D3), a secosteroid hormone that is the active form of vitamin D. It can also be converted into 24-hydroxycalcidiol in the kidneys via 24-hydroxylation[2-3].
In medicine, a 25-hydroxy vitamin D blood test is used to determine how much vitamin D is in the body. The blood concentration of 25-hydroxy vitamin D is considered the best indicator of vitamin D status[4]. This test can be used to diagnose vitamin D deficiency, and it is indicated in patients with high risk for vitamin D deficiency and when the results of the test would be used as supporting evidence for beginning aggressive therapies. Patients with osteoporosis, chronic kidney disease, malabsorption, obesity, and some other infections may be high risk and thus have greater indication for this test. Although vitamin D deficiency is common in some populations including those living at higher latitudes or with limited sun exposure, the 25(OH) D test is not indicated for entire populations. Physicians may advise low risk patients to take over-the-counter vitamin D in place of having screening[5-8].
It is the most sensitive measure, though experts have called for improved standardization and reproducibility across different laboratories[4]. According to MedlinePlus, the normal range of calcifediol is 30.0 to 74.0 ng/mL. The normal range varies widely depending on several factors, including age and geographic location. A broad reference range of 20–150 nmol/L (8-60 ng/mL) has also been suggested[9], while other studies have defined levels below 80 nmol/L (32 ng/mL) as indicative of vitamin D deficiency[10].

PRINCIPLE OF THE TEST

The 25-OH Vitamin D assay is a competitive chemiluminescence immunoassay.
The 25-OH Vitamin D assay is a two-incubation chemiluminescence immunoassay for the quantitative determination of total 25-OH vitamin D in human serum. In the first incubation, the 25-OH vitamin D is dissociated from its binding protein by the displacing reagent, and binds to the 25-OH vitamin D antibody on the magnetic microbeads forming an antibody-antigen complex. Following a second incubation, the 25-OH Vitamin D labeled ABEI are added. The rest unbound material is removed during a wash cycle. Subsequently, the Starter 1+2 are added to initiate a flash chemiluminescent reaction. The resulting chemiluminescent reaction is measured as relative light units (RLUs), which is inversely proportional to the concentration of 25-OH Vitamin D present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130211004M)	50 tests (REF: 130611004M)
Magnetic Microbeads	Magnetic microbeads coated with 25-OH Vitamin D monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and 25-OH Vitamin D antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Calibrator High	Containing BSA and 25-OH Vitamin D antigen, NaN_3 (<0.1%).	3.0 mL	2.0 mL
Displacing Reagent	Acidic buffer.	6.5 mL	4.0 mL
ABEI Label	25-OH Vitamin D antigen labeled with ABEI.	12.5 mL	7.0 mL
Internal Quality Control	Containing BSA and 25-OH Vitamin D antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against the NIST SRM 972a.
Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If controls lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value, refer to ***25-OH Vitamin D (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate substance.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed once. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details discussion of onboard sample storage constraints.
- Specimens removed from the separator, red blood cells or clot may be stored up to 6 hours at 2-8°C, and stored up to 30 days frozen at -20°C or colder.
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of 25-OH Vitamin D is 100 µL.

WARNING AND PRECAUTIONS FOR USERS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION**: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the Reagent Kit on the system for the first time, the Reagent Kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

LIMITATIONS

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement.
- Any therapeutical decision should also be taken on a case-by-case basis.
- Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

Calculation of Results

The analyzer automatically calculates the 25-OH Vitamin D concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/mL. For further information, please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

Factors such as dietary intake, race, UV exposure, season, and age are all known to affect the normal levels of 25-OH Vitamin D.

A review of the literature suggests the following ranges for the classification of 25-OH Vitamin D status:

Vitamin D status	25-OH Vitamin D
Deficiency	< 10 ng/mL
Insufficiency	10-29 ng/mL
Sufficiency	30-100 ng/mL
Toxicity	>100 ng/mL

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own expected ranges.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the 25-OH Vitamin D assay was determined as described in the CLSI EP5-A2. 3 controls and 3 human serum pools containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(ng/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(ng/mL)	%CV	SD(ng/mL)	%CV	SD(ng/mL)	%CV
Serum Pool 1	15.897	0.704	4.43	1.103	6.94	1.308	8.23
Serum Pool 2	45.870	1.954	4.26	2.115	4.61	2.879	6.28
Serum Pool 3	97.569	3.258	3.34	0.843	0.86	3.365	3.45
Control 1	32.095	1.527	4.76	1.218	3.79	1.953	6.09
Control 2	59.923	2.129	3.55	2.203	3.68	3.064	5.11
Control 3	119.874	2.559	2.13	4.086	3.41	4.821	4.02

Limit of Blank (LoB)

The LoB for the 25-OH Vitamin D assay is 3.0 ng/mL.

Measuring Range

3.0-150 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <3.0 ng/mL. Values above the measuring range are reported as >150 ng/mL.

Recovery

The 25-OH Vitamin D assay has a mean recovery of 90%-110%. Two different levels of triiodothyronine were spiked into three samples resulted in the following data:

Sample	Added (ng/mL)	Observed (ng/mL)	**%**Recovery
S1	-	21.055	/
	25.26	47.262	103.75
	63.08	85.018	101.40
S2	-	46.328	/
	25.26	71.348	99.05
	63.08	108.966	99.30
S3	-	79.922	/
	25.26	105.156	99.90
	63.08	142.781	99.65

Method Comparison

A total of 100 clinical samples in the range of 4.27 and 146.79 ng/mL were tested by the 25-OH Vitamin D assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as:

$y=0.964x+1.223$, $r^2=0.981$.

Analytical Specificity
The specificity data of the assay was obtained by spiking up to 100 ng/mL of the potential cross-reactant to serum samples at the indicated concentrations. The result was listed in the table below:

Cross Reactant	Cross-reactivity
3-epi-25 OH Vitamin D3	2.04%

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:

- Bilirubin 20 mg/dL
- Hemoglobin 200 mg/dL
- Triglyceride 549 mg/dL

REFERENCES

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code