

# MAGLUMI 25-OH Vitamin D (CLIA)



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## **FOR PROFESSIONAL USE ONLY**

Store at 2-8°C



COMPLETELY READ THE INSTRUCTIONS BEFORE  
PROCEEDING



## **SYMBOLS EXPLANATIONS**



Authorized Representative in Europe



Manufacturer



Attention. See Instructions For Use



Contents of kit



In vitro diagnostic medical device  
(In vitro diagnostic use)



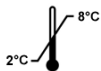
Lot number



Catalogue Code



Expiry date (Use by...)



Temperature limitation  
( store at 2-8°C)



Number of tests



Keep away from sunlight



Keep upright

## **INTENDED USE**

The kit has been designed for the quantitative determination of 25-OH Vitamin D in human serum.

The method can be used for samples over the range of 0-150ng/ml.

The test has to be performed on the MAGLUMI chemiluminescence immunoassay (CLIA) fully auto analyzer (Including MAGLUMI 1000, MAGLUMI 2000, MAGLUMI 2000 Plus and new developed models).

## **SUMMARY AND EXPLANATION OF THE TEST**

Vitamin D is a group of fat-soluble secosteroids. In humans, vitamin D is unique both because it functions as a prohormone and because the body can synthesize it (as vitamin D<sub>3</sub>) when sun exposure is adequate (hence its nickname, the "sunshine vitamin").

Several forms (vitamers) of vitamin D exist. The two major forms are vitamin D<sub>2</sub> or ergocalciferol, and vitamin D<sub>3</sub> or cholecalciferol, vitamin D without a subscript refers to either D<sub>2</sub> or D<sub>3</sub> or both. These are known collectively as calciferol. Vitamin D<sub>2</sub> was chemically characterized in 1932. In 1936, the chemical structure of vitamin D<sub>3</sub> was established and resulted from the ultraviolet irradiation of 7-dehydrocholesterol.

Vitamin D<sub>2</sub> is a derivative of ergosterol, a membrane sterol named for the ergot fungus, which is produced by some organisms of phytoplankton, invertebrates, and fungi. The vitamin ergocalciferol (D<sub>2</sub>) is produced in these organisms from ergosterol in response to UV irradiation. D<sub>2</sub> is not produced by land plants or vertebrates, because they lack the precursor ergosterol. The biological fate for producing 25(OH)D from vitamin D<sub>2</sub> is expected to be the same as for D<sub>3</sub>.

Low blood calcidiol (25-hydroxy-vitamin D) can result from avoiding the sun. Deficiency results in impaired bone mineralization, and leads to bone softening diseases including:

We often use dose of 5000IU / month to 50000IU / week of vitamin D<sub>3</sub>(or D<sub>2</sub>) to treat the Vitamin D deficiency, fortified foods and nutritional supplements may contain some form of VD, in order to ensure accurate assessment of the total content of vitamin D, vitamin D must be including all forms of vitamin D<sub>3</sub>, D<sub>2</sub> and metabolites measured.

Recent studies have confirmed that children's serum under 1 year old may exist 25-OH vitamin D in non-active 3 – epimer form, the test kit should be one of the important properties for the detection of only active ingredients, such as 25-OH vitamin D D<sub>3</sub> and D<sub>2</sub>, but not inactive 3 - epimer of interference.

## **PRINCIPLE OF THE TEST**

Competitive immunoluminometric assay:

Use a purified 25-OH Vitamin D antigen to label ABEI, and use 25-OH Vitamin D monoclonal antibody to label FITC. Sample, Calibrator or Control with Displacing reagent, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37°C, forming antibody-antigen complexes; After sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Then add ABEI Label, incubation and washing for the 2nd time, sample antigen and ABEI labeled antigen compete to combine with FITC labeled monoclonal antibody, forming antibody-antigen complexes. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of 25-OH Vitamin D present in controls or samples.

**CONT****KIT COMPONENTS****Material Supplies**

Reagent Integral for 100 determinations	
<b>Nano magnetic microbeads:</b> TRIS buffer, 1.2% (W/V), 0.2%NaN <sub>3</sub> , coated with sheep anti-FITC polyclonal antibody.	2.5ml
<b>Calibrator Low:</b> bovine serum, 0.2%NaN <sub>3</sub> .	3.0ml
<b>Calibrator High:</b> bovine serum, 0.2%NaN <sub>3</sub>	3.0ml
<b>Displacing reagent:</b> acidic buffer	6.5ml
<b>FITC label:</b> 25-OH Vitamin D monoclonal antibody labeled FITC contains BSA, 0.2%NaN <sub>3</sub> .	12.5ml
<b>ABEI label:</b> 25-OH Vitamin D antigen labeled ABEI, contains BSA, 0.2%NaN <sub>3</sub> .	22.5ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
<b>Internal Quality Control:</b> containing BSA, 0.2%NaN <sub>3</sub> . (target value refer to Quality Control Information data sheet)	2.0ml

**Accessories Required But Not Provided**

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

**Preparation of the Reagent Integral**

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!) Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

**Do not interchange integral component from different reagents or lots!**

**Storage and Stability**

- Sealed: Stored at 2-8 °C until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.



- Keep upright for storage.



- Keep away from direct sunlight.

**CALIBRATION AND TRACEABILITY****1) Traceability**

To perform an accurate calibration, we have provided the test calibrators standardized against the SNIBE internal reference substance.

Calibrators in the Reagent Kit are from Sigma.

**2) 2-Point Recalibration**

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

**3) Frequency of Recalibration**

- After each exchange of lots (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the MAGLUMI Fully Auto analyzer.
- If controls are beyond the expected range.

**SPECIMEN COLLECTION AND PREPARATION**

Sample material: serum

Collect samples using standard procedures.

Store at 2-8 °C: 24 hours, for lo

below -20 °C

Avoid repeated freezing and thawing cycles, stored samples should be thoroughly mixed prior to use (Vortex mixer).

Please ask local representative of SNIBE for more details if you have any doubt.

**Vacuum Tubes**

- Blank tubes are recommended type for collecting samples.
- Please ask SNIBE for advice if special additive must be used in sample collecting.

**Specimen Conditions**

- Do not use specimens with the following conditions:
  - heat-inactivated specimens;
  - Cadaver specimens or body fluids other than human serum;
  - Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- 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 clot forms, the presence of fibrin may cause erroneous results.

**Preparation for Analysis**

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed discussion of onboard sample storage constraints.

**Storage**

- If testing will be delayed for more than 8 hours, remove serum or plasma from the serum or plasma separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 24 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

**Shipping**

Before shipping specimens, it is recommended that specimens be

removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

## WARNING AND PRECAUTIONS FOR USERS



- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. 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.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24, 2000, p.2143). A minimum of one hour at 121 °C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens<sup>13</sup>. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

### Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

## TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the MAGLUMI Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the MAGLUMI 103120328-v1.0-EN

## Chemiluminescence Analyzer Operating Instructions.

100µl	Sample, calibrator or controls
+100µl	FITC Label
+50µl	Displacing reagent
+20µl	Nano magnetic microbeads
20 min	Incubation
400µl	Cycle washing
+200µl	ABEI Label
10 min	Incubation
400µl	Cycle washing
3 s	Measurement

## DILUTION

Samples with concentrations above the measuring range can be diluted. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

Availability of sample dilution by analyzer please refers to the MAGLUMI analyzer user software program. Dilution settings please follow MALGUMI analyzer operating instructions.

## QUALITY CONTROL

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

## LIMITATIONS OF THE PROCEDURE

### 1) Limitations

Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.

Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results.

Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

### 2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin<0.06mg/ml, haemoglobin<16mg/dl or triglycerides<12.5mg/ml.

### 3) HAMA

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

### 1) 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 MAGLUMI Chemiluminescence Analyzer Operating Instructions.

### 2) Interpretation of Results

- Results of study in clinical centers with group of individuals

were

Vitamin D status	25-OH Vitamin D concentration	
	ng/ml	nmol/L
lack of V-D	<10	<25
insufficiency	10-30	25-75
normal	30-100	75-250
toxicosis	>100	>250

- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

## PERFORMANCE CHARACTERISTICS

### 1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	11.28	0.39	3.45%
Level 2	33.49	1.01	3.01%
Level 3	81.06	2.57	3.17%

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	10.77	0.67	6.25%
Level 2	33.65	2.03	6.04%
Level 3	83.69	5.13	6.13%

### 2) Analytical Sensitivity

The sensitivity is defined as the concentration of 25-OH Vitamin D equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 3ng/ml.

### 3) Specificity

The specificity of the 25-OH Vitamin D assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
3-epi-25 OH Vitamin D3	100 ng/ml	0.3%

### 4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% -110%.

Expected	Mean Measuring	Recovery
50.1 ng/ml	51.3 ng/ml	102%

### 5) Linearity

Use 25-OH Vitamin D calibrator to prepare the six-point standard curve, measuring all points' RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9800.

Calibrator Point	Concentration ng/ml	Absolute linear correlation coefficient (r)
A	0	
B	10	r=0.9890
C	20	
D	40	
E	80	
F	150	

## 6) Method comparison

A comparison of MAGLUMI 25-OH Vitamin D (y) with a commercially available 25-OH Vitamin D test (x) using clinical samples gave the following correlations (ng/ml):

Linear regression

$$y = 0.97x + 60.0$$

$$r = 0.976$$

$$S_{y.x} = 18.2$$

Number of samples measured: 200

The sample concentrations were between 12 and 130 ng/ml.

## REFERENCES

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