Snibe Diagnostic

CE

REF 130201001M: 100 tests
130601001M: 50 tests

MAGLUMI® Ferritin (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Ferritin 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

Ferritin is a globular protein complex consisting of 24 protein subunits forming a nanocage with multiple metal–protein interactions. It is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form[1]. Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including algae, bacteria, higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload. Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body, hence serum ferritin is used as a diagnostic test for iron-deficiency anemia[2].

Ferritin serves to store iron in a non-toxic form, to deposit it in a safe form, and to transport it to areas where it is required[3]. The function and structure of the expressed ferritin protein varies in different cell types. This is controlled primarily by the amount and stability of Messenger RNA (mRNA). mRNA concentration is further tweaked by changes to how it is stored and how efficiently it is transcribed. The presence of iron itself is a major trigger for the production of ferritin, with some exceptions (such as the yolk ferritin of the gastropod Lymnaea, which lacks an iron-responsive unit) [4-5]. Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen species via the Fenton Reaction. Hence vertebrates evolve an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells, iron is stored in a protein complex as ferritin or hemosiderin[6]. As ferritin accumulates within cells of the reticuloendothelial system, protein aggregates are formed as hemosiderin. Iron in ferritin or hemosiderin can be extracted for release by the RE cells although hemosiderin is less readily available.

Ferritin concentrations increase drastically in the presence of an infection or cancer. Endotoxins are an up-regulator of the gene coding for ferritin, thus causing the concentration of ferritin to rise. By contrast, organisms such as Pseudomonas, although possessing endotoxin, cause serum ferritin levels to drop significantly within the first 48 hours of infection. Thus, the iron stores of the infected body are denied to the infective agent, impeding its metabolism[7]. Serum ferritin levels are measured as part of the iron studies workup for iron-deficiency anemia. The ferritin levels measured usually have a direct correlation with the total amount of iron stored in the body. However, ferritin levels may be artificially high in cases of anemia of chronic disease where ferritin is elevated in its capacity as an inflammatory acute phase protein and not as a marker for iron overload[1].

PRINCIPLE OF THE TEST

The Ferritin assay is a two-step sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable) and magnetic microbeads coated with anti-Ferritin monoclonal antibody are mixed thoroughly and incubated, and then perform a wash cycle. Then add ABEI labeled with anti-Ferritin monoclonal antibody are mix thoroughly and incubate to form sandwich complexes. After precipitation in a magnetic field, decant the supernatant, and then perform another wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of ferritin present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130201001M)	50 tests (REF: 130601001M)
Magnetic Microbeads	Magnetic microbeads coated with anti-Ferritin monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Ferritin antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Ferritin antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	12.5 mL	7.0 mL
ABEI Label	Anti-Ferritin monoclonal antibody labeled ABEI, containing BSA, NaN_3 (<0.1%).	22.5 mL	12.5 mL
Diluent	0.9%NaCl.	25.0 mL	15.0 mL
Internal Quality Control	Ferritin antigen, containing bovine serum, 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 WHO 3rd International Standard 94/572.
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 4 weeks 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 ***Ferritin (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 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 matter.
- 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 repeated freezing and thawing. The serum sample can be frozen and thawed for once. Stored samples should be thoroughly mixed prior to use (Vortex mixer). Frozen specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing. Please ask local representative of SNIBE for more derails if you have any doubt.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care should be taken to transfer only the clarified specimen without the lipaemic 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 of onboard sample storage constraints.
- Specimens removed from the separator, red blood cells or clot may be stored up to 7 days at 2-8°C, and stored up to 12 months frozen at -20°C or colder.
- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator. 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 ferritin is 20 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

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.
The automatic sample dilution is available after dilution settings are done in the MAGLUMI and Biolumi series Fully-auto chemiluminescence immunoassay analyzer user software. Please refer to the corresponding Analyzer Operating Instructions.

High-Dose Hook

For the Ferritin assay, no high dose hook effect was observed when samples containing Ferritin up to 100,000 ng/mL.

LIMITATIONS

- A skillful operation and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions should be followed exactly and careful operation should be used to obtain valid results. Any modification of the procedure is likely to alter the results.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or received immunotherapy may contain human anti-mouse antibodies (HAMA), which may result in falsely elevated or decreased values. Moreover, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples as well. Additional clinical or diagnostic information may be required to determine patient status.

RESULTS

Calculation of Results

The analyzer automatically calculates the Ferritin 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

The expected values for the Ferritin assay were obtained by testing 292 apparently healthy individuals (132 males and 160 females) in China, and gave the following interval values listed below:
Males: 25-350 ng/mL (2.5^{th}-97.5^{th} percentiles).
Females: 13-232 ng/mL (2.5^{th}-97.5^{th} percentiles).
Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own expected ranges.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the Ferritin assay was determined as described in the CLSI EP5-A2. 3 human serum pools and 2 controls 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)	Within-Run		Between-Run		Total	
	(N=80)	SD(ng/mL)	%CV	SD(ng/mL)	%CV	SD(ng/mL)	%CV
Serum Pool 1	29.704	1.494	5.03	1.988	6.69	2.487	8.37
Serum Pool 2	299.046	11.536	3.86	3.407	1.14	12.028	4.02
Serum Pool 3	899.937	27.766	3.09	13.378	1.49	32.354	3.60
Control 1	57.949	2.622	4.53	3.190	5.50	4.130	7.13
Control 2	1191.215	44.617	3.75	24.092	2.02	50.706	4.26

Limit of Blank (LoB)

The LoB for the Ferritin assay is 0.2 ng/mL.

Limit of Detection (LoD)

The LoD for the Ferritin assay is 0.5 ng/mL.

Measuring Range

0.2-3000 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.2 ng/mL. Values above the measuring range are reported as >3000 ng/mL.

Linearity

The assay is linear between 0.5 ng/mL and 3000 ng/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by spiking a serum sample containing Ferritin 3300 ng/mL with a serum sample free of Ferritin (0.0 ng/mL).The mean sample recovery ranged from 90% to 110%.

Method Comparison

A total of 100 samples in the range of 1.96 to 2838.76ng/mL were tested by the Ferritin assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: $y=0.991x-0.354$, $r^2=0.991$.

Analytical Specificity

The specificity data of the assay was obtained by adding these cross-reactant at the indicated concentrations to serum samples at the indicated concentrations. The Cross-reactivity of the assay was shown in the following table:

Compound	Concentration	%Cross reactivity
Human liver ferritin	850 ng/mL	82
Human spleen ferritin	450 ng/mL	50
Human heart ferritin	500 ng/mL	1

Endogenous Interference

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

- Bilirubin 65 mg/dL
- Hemoglobin 2000 mg/dL
- Triglyceride 3300 mg/dL

REFERENCES

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EC REP

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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