Snibe Diagnostic

CE

REF 130203005M: 100 tests
130603005M: 50 tests

MAGLUMI® FT3 (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescent immunoassay for the quantitative determination of Free Triiodothyronine (FT3) 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

Triiodothyronine, also called T3, is a thyroid hormone with a molecular weight of 651daltons and a half-life in serum of 1.5 days, which affects almost every physiological process in the body such as growth and development, metabolism, body temperature and heart rate[1,2].

The thyroid gland exclusively synthesizes T4 and produces approximately 20% of T3. The majority of T3 is generated by 5′-deiodination catalyzed by type I and type II T4-5′-deiodinases in peripheral tissue[3]. T3 circulates in the bloodstream as an equilibrium mixture of free and serum bound hormone. Free T3 (fT3) is the unbound and biologically active form, of which only approximately 0.04% of total T3 are available in circulation as free hormones and are considered the biologically active forms because the free hormone is accessible to peripheral tissue[4-6].

Thyroxine-binding globulin (TBG) is a high affinity, but low-concentration, binding protein that binds approximately 80% of T3 and 75% of T4. The remainder of circulating T3 and T4 are bound to the low affinity proteins albumin and transthyretin. Changes in binding protein concentrations occur in a number of conditions and can significantly affect the total thyroid hormone concentrations[7,8].

Free T3 is an important test for diagnosing or monitoring hyperthyroidism. Free T3 is not suitable for diagnosing hypothyroidism as some hypothyroid patients have reduced free T4 and elevated TSH, but a normal free T3. However, clinical studies have shown that free T3 is a better indicator of hyperthyroidism than total T3, as it is independent of thyroid hormone binding protein concentrations. Therefore free T3 is a useful tool in clinical routine diagnostics for the assessment of thyroid status and supporting the differential diagnosis of thyroid disorders, and to identify patients with T3 thyrotoxicosis[9,10].

Pregnancy or the use of oral contraceptives or estrogens may increase total T3 levels, while concentrations of free T3 remain basically unchanged.

PRINCIPLE OF THE TEST

The FT3 assay is a competitive chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), ABEI-labeled anti-T3 monoclonal antibody, buffer are mixed thoroughly and incubated, and then the solution of the magnetic microbeads coated with T3 antigens is added and incubated. T3 present in the serum sample (or calibrator/control, if applicable) competes with T3 antigen immobilized on the magnetic microbeads for a limited number of binding sites on the ABEI-labeled anti-T3 antibody. After precipitation in a magnetic field, decant the supernatant, and then perform a 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 inversely proportional to the concentration of FT3 present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Component	Contents	100 tests (REF: 130203005M)	50 tests (REF: 130603005M)
Magnetic Microbeads	Magnetic microbeads coated with purified T3 antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and T3 antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Containing BSA and T3 antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
ABEI Label	Anti-T3 monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	6.5 mL	4.0 mL
Internal Quality Control	Containing BSA and T3 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 USP (United States Pharmacopeia).

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 exchange 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 ***FT3 (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, commercial 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 support provider or distributor 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 matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate matter 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 one time. 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, cells or clot may be stored up to 24 hours at 2-8°C. Freeze samples at or below -20°C if the sample is not assayed within 24 hours.
- 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 FT3 is 40 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 of 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 sample.
- 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. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION
Samples for FT3 determinations cannot be diluted, as T3 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium.

LIMITATIONS
- A skillful operation and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions must be followed exactly and careful operation must 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[11-13]. Serum FT3 levels alone give no evidence of the presence or absence of thyroid disease. They must always be interpreted in context with the clinical symptom and other diagnostic procedures.
- Certain drugs and clinical conditions are known to alter Free T3 concentrations in vivo. For example, transient elevations in FT3 concentrations may be observed following administration of drugs (e.g. furosemide, phenylbutazone, probenecid, sulindac and fenclofenac) that displace T3 from their binding proteins. For additional information, refer to one of the published summaries[9,14-17].
- Patients receiving heparin can have biased free T3 results as heparin stimulated the production of non-esterified fatty acids, which displace T3 from albumin. Heparin can also arise from indwelling cannulas containing a heparin-solution[9].

RESULTS
Calculation of Results

The analyzer automatically calculates the FT3 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 pg/mL. For further information please refer to the corresponding Analyzer Operating Instructions.
Conversion factor: pg/mLx1.54=pmol/L.

Interpretation of Results

The expected range for the FT3 assay was obtained by testing 240 apparently healthy individuals in China, and gave the following reference interval listed below:
2.0-4.2 pg/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 establish its own expected ranges.

PERFORMANCE CHARACTERISTICS
Precision

Precision for the FT3 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 result is summarized in the following table:

Sample	Mean(pg/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(pg/mL)	%CV	SD(pg/mL)	%CV	SD(pg/mL)	%CV
Serum Pool 1	2.074	0.096	4.63	0.127	6.12	0.159	7.67
Serum Pool 2	4.603	0.165	3.59	0.156	3.39	0.227	4.93
Serum Pool 3	18.407	0.673	3.66	0.325	1.77	0.748	4.06
Control 1	3.102	0.137	4.42	0.140	4.51	0.196	6.32
Control 2	20.392	0.605	2.97	0.628	3.08	0.871	4.27
Control 3	40.550	1.069	2.64	1.044	2.57	1.494	3.68

Limit of Blank (LoB)

The LoB for the FT3 assay is 0.2 pg/mL.

Limit of Detection (LoD)

The LoD for the FT3 assay is 0.4 pg/mL.

Measuring Range

0.2-50 pg/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 pg/mL. Values above the measuring range are reported as >50 pg/mL.

Method Comparison

A total of 113 clinical samples in the range of 0.357-19.239 pg/mL were tested by the FT3 assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: $y=0.970x+0.2338$, $r^2=0.9506$.

Analytical Specificity

The FT3 assay was evaluated for interference consistent.
The Cross-reactivity of the FT3 assay with a cross reactant can be expressed as the ratio of
- The amount of T3 required to displace 50% of the maximally bound labeled T3 from the anti-T3 antibody, and
- The amount of the cross-reactant to give the same 50% displacement.

The results were listed in the following table:

Cross Reactant	%Cross Reactivity
L-Triiodothyronine	<0.01
Diiodotyrosine	<0.01
Monoiodotyrosine	<0.01
3,5-Diiodo-L-thyronine	<0.01

Drugs up to the following concentrations did not interfere with the assay:
- Phenylbutazone 15.0 mg/dL
- Sodium salicylate 50.0 mg/dL
- Aspirin 50.0 mg/dL
- Ibuprofen 50.0 mg/dL

- Acetaminophen 20.0 mg/dL
- Phenytoin 5.0 mg/dL
- Amiodarone 20.0 mg/dL
- Propylthiouracil 30.0 mg/dL

Endogenous Interference

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

- Bilirubin 33 mg/dL
- Triglyceride 4300 mg/dL
- Hemoglobin 2000 mg/dL
- Total Protein 12 g/dL
- Rheumatoid factor 620 IU/mL
- HAMA 1232 ng/mL

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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