Snibe Diagnostic

CE REF 130203003M: 100 tests
130603003M: 50 tests

MAGLUMI® T3 (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Triiodothyronine (T3) 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, which affects almost every physiological process in the body such as growth and development, metabolism, body temperature and heart rate[1].

The thyroid gland exclusively synthesizes thyroxine (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. Specifically, approximately 65% of peripheral-derived T3 is produced by type II deiodinase, a microsomal enzyme with activity in the brain, muscle, pituitary, and placenta; the remaining 35% is derived from type I deiodinase activity, a plasma membrane enzyme located in the thyroid, liver, and kidney[2]. The majority of circulating thyroid hormones are bound to serum proteins, allowing for their transport and extension of half-life; however, carrier protein binding makes thyroid hormones unavailable to tissues and thus protein-bound T3 and T4 are considered inactive. Thyroxine-binding globulin (TBG) is a high-affinity but low-concentration binding protein that binds approximately 80% of T3 and 75% of T4[3]. The remainder of circulating T3 and T4 are bound to the low affinity proteins like albumin and transthyretin. Changes in binding protein concentrations occur in a number of conditions and can significantly affect the total thyroid hormone concentrations. Only approximately 0.04% of total T3 and 0.02% of T4 are available in circulation as free hormones and are considered the biologically active forms because the free hormone is accessible to peripheral tissue[4].

T3 concentrations may be altered in conditions affecting the capacity of the thyroid hormone binding proteins, e.g. pregnancy. A fall in T3 concentrations of up to 50% is known to occur in a variety of clinical situations, including acute and chronic disease[5,6]. Total serum T3 determination serves as a great important role in diagnosing thyroid disorders, which is elevated in most classical causes of hyperthyroidism, and decreased in primary hypothyroid disease such as neonatal hypothyroidism or secondary hypothyroidism[7].

PRINCIPLE OF THE TEST

The T3 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 forming immuno-complexes. 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 T3 present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Component	Contents	100 tests (REF: 130203003M)	50 tests (REF: 130603003M)
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	0.1%ANS, containing BSA, NaN_3 (<0.1%).	6.5 mL	4.0 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 ***T3 (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 support provider 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 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 frozen and thawed only 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 of 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 T3 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 therefore should 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

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

- 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.

- Normal T3 concentrations do not necessarily reflect a normal-thyroid state. Certain thyroid disorders (such as latent hypo- or hyperthyroidism, compensatory T3 over secretion in iodine deficiency, TBG over secretion) may also be associated with euthyroid T3 levels.
- The clinical evaluation of serum findings must take into consideration including age- or pregnancy-related differences as well as a potential influence of exogenously administered thyroid hormones, contraceptives, steroids, salicylates, diphenylhydantoin or other drugs as well as changes of the binding capacities of serum proteins for thyroid hormones.
- 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[8-10]. Additional clinical or diagnostic information may be required to determine patient status.
- Binding protein anomalies seen with FDH (Familial Dysalbuminemic Hyperthyroxinemia), for example, may cause values which, while characteristic of the condition, deviate from the expected results[11].
- For diagnostic purposes, the results should be interpreted in the context of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

RESULTS

Calculation of Results

The analyzer automatically calculates the T3 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.
Conversion factor: ng/mLx1.54=nmol/L.

Interpretation of Results

The expected range for the T3 assay was obtained by testing 212 apparently healthy individuals in China, and gave the following expected value:
0.69-2.15 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 establish its own expected ranges.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the T3 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(ng/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(ng/mL)	%CV	SD(ng/mL)	%CV	SD(ng/mL)	%CV
Serum Pool 1	1.155	0.040	3.46	0.011	0.95	0.046	3.98
Serum Pool 2	3.517	0.095	2.70	0.120	3.41	0.153	4.35
Serum Pool 3	4.760	0.102	2.14	0.064	1.34	0.120	2.52
Control 1	0.812	0.051	6.28	0.021	2.59	0.059	7.27
Control 2	2.298	0.096	4.18	0.138	6.01	0.168	7.31
Control 3	4.582	0.112	2.44	0.090	1.96	0.143	3.12

Limit of Blank (LoB)

The LoB for the T3 assay is 0.06 ng/mL.

Limit of Detection (LoD)

The LoD for the T3 assay is 0.2 ng/mL.

Measuring Range

0.06-10 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.06 ng/mL. Values above the measuring range are reported as >10 ng/mL.

Recovery

The T3 assay has a mean recovery of 90%-110%. Two different levels of triiodothyronine were spiked into three samples, the results were listed in the following table:

Sample	Amount added (ng/mL)	Observed (ng/mL)	**%**Recovery
S1	-	0.709	
	1.50	2.126	94.50
	3.20	4.098	105.90
S2	-	2.093	
	1.50	3.584	99.40
	3.20	5.186	96.65
S3	-	4.486	
	1.50	5.959	98.15
	3.20	7.733	101.45

Method Comparison

A total of 160 clinical samples in the range of 0.198 to 9.587 ng/mL were tested by the T3 assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=1.056x-0.0731, r^2=0.9504.

Analytical Specificity

The Cross-reactivity of the T3 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
Diiodotyrosine	<0.01
Monoiodotyrosine	0.04
Thyroxine	0.01

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

- Phenylbutazone 15.0 mg/dL
- Sodium salicylate 10.0 mg/dL
- Aspirin 10.0 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 35 mg/dL
- Triglyceride 2000 mg/dL
- Hemoglobin 1800 mg/dL
- Total Protein 12 g/dL
- Rheumatoid factor 620 IU/mL
- HAMA 1232 ng/mL

REFERENCES

1. Bowen, R. (2010-07-24). "Physiologic Effects of Thyroid Hormones". Colorado State University. Retrieved 2013-09-29.
2. Bianco AC & Kim BW. Deiodinases: implications of the local controlof thyroid hormone action. Journal of Clinical Investigation 2006 116 (2571–2579).
3. Schussler GC. The thyroxine-binding proteins. Thyroid 2000 10(141–149).
4. Ekins R. The free hormone hypothesis and measurement of freehormones. Clinical Chemistry 1992 38 (1289–1293).
5. Cavalieri RR &Rapoport B.: Impaired Peripheral Conversion of Thyroxine to Triiodothyronine. Ann Rev Med 28: 57-65 (1977).
6. Tunbridge WMG & Hall R.: Thyroid Function in Pregnancy. Clinics in Obstetrics and Gynaecology 2: 381-393 (1975).
7. Gornall, AG, Luxton, AW, Bhavnani, BR. Endocrine disorders. In Applied Biochemistry of Clinical Disorders, 1986; 305-318.
8. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res 1985; 45(2):879-885.
9. Kricka,L. Interference in immunoassays-still a threat. Clin Chem 2000; 46:1037-1038.
10. Primus FJ, Kelley EA, Hansen HJ, et al. “Sandwich”-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. Clin Chem 1988; 34(2):261-264.
11. Wada N, Chiba H, Shimizu C, et al. A Novel Missense Mutation in Codon 218 of the Albumin Gene in a Distinct Phenotype of Familial Dysalbuminemic Hyperthyroxinemia in a Japanese Kindred. Journal of Clinical Endocrinology and Metabolism 1997; 82(10):3246-3250.

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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