Snibe Diagnostic

CE REF 130203002M: 100 tests
130603002M: 50 tests

MAGLUMI® T4 (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Thyroxine (T4) 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

Thyroxine (3,5,3',5'-tetraiodothyronine, T4) , a derivative of the amino acid tyrosine containing 4 iodines, is the major form of the thyroid hormones in the blood, which plays a vital role in modulating the proper development and differentiation of all cells of the human body and also regulating basal metabolic rate, protein synthesis, long bone growth and neural maturation. Thyroxine is formed and stored in the thyroid follicles as the precursor thyroglobulin, which then is cleaved by proteolytic enzyme to produce thyroxine and released from thyroid gland[1,2].

In the circulation, approximately 99.98% of T4 is bound to plasma proteins: thyroxine binding globulin (TBG) 60%-70%, transthyretin of thyroxine binding prealbumin (TTR/TBPA) 15%-30%, and albumin 10%, however a very small fraction nearly 0.03% of T4 is unbound and biologically active[3]. T4 could be converted to the active T3 with one less iodine atom per molecule in the cells by deiodinases, while T3 has greater biological potency and is more active than T4 although it is present in less quantity than T4[4]. As the concentration of binding proteins can be easily subject to several exogenous and endogenous effects like genetic, non-thyroidal illness, pregnancy, and changes in these factors have a pronounced effect on the serum concentrations of total T4 and total T3, therefore compromising the diagnosis of disease activity in the thyroid gland[5]. Drugs such as phenylbutazone, salicylates which compete for protein binding sites could result in reduced measurement of T4 and then erroneous interpretation of the thyroid function[6,7].

Clinically, the determination of total T4 is an important indicator in diagnosing and confirming the status of thyroid disorders such as hyperthyroidism, primary and secondary hypothyroidism[8]. However, it can be sometimes inadequate, and more reliable indications as well as maximum diagnostic accuracy may be improved by use of total T4 test in conjunction with additional test such as TSH, Free T4, Free T3 and total T3.

PRINCIPLE OF THE TEST

The T4 assay is a competitive chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), displacing solution, ABEI-labeled anti-T4 monoclonal antibody, buffer and T4 antigen-coated magnetic microbeads are mixed thoroughly and incubated. T4 present in the serum sample (or calibrator/control, if applicable) competes with T4 antigen immobilized on the magnetic microbeads for a limited number of binding sites on the ABEI-labeled anti-T4 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 T4 present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130203002M)	50 tests (REF: 130603002M)
Magnetic Microbeads	Magnetic microbeads coated with purified T4 antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and T4 antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Containing BSA and T4 antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Displacing Solution	0.4M NaOH.	6.0 mL	6.0 mL
Buffer	0.1%ANS, containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
ABEI Label	Anti-T4 monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	6.5 mL	4.0 mL
Internal Quality Control	Containing BSA and T4 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) Standards.

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 ***T4 (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 supporter 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 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 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 detailed 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 T4 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 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. 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.
- In pregnancy, the Total T4 results may be incorrect, i.e., falsely-low. This assay should not be used as the only marker for thyroid disease evaluation during pregnancy. To ensure maximum diagnostic accuracy, thyroid status in pregnant women should be determined using thyroid function tests such as TSH, Free T4, and clinical evaluation by the physician.

RESULTS

Calculation of Results

The analyzer automatically calculates the T4 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/mLx1.287=nmol/L.

Interpretation of Results

The expected range for the T4 assay was obtained by testing 231 apparently healthy individuals in China, and gave the following expected value:
52-127 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 T4 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	45.249	2.270	5.02	2.068	4.57	3.071	6.79
Serum Pool 2	79.879	2.829	3.54	2.141	2.68	3.547	4.44
Serum Pool 3	129.381	3.911	3.02	2.782	2.15	4.800	3.71
Control 1	60.150	2.794	4.65	1.618	2.69	3.229	5.37
Control 2	120.767	4.103	3.40	3.102	2.57	5.144	4.26
Control 3	164.767	4.275	2.59	4.264	2.59	6.038	3.66

Limit of Blank (LoB)

The LoB for the T4 assay is 1.0 ng/mL.

Limit of Detection (LoD)

The LoD for the T4 assay is 1.5 ng/mL.

Measuring Range

1.0-300 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <1.0 ng/mL. Values above the measuring range are reported as >300 ng/mL.

Recovery

The T4 assay has a mean recovery of 100%±10%. Two different levels of thyroxine were added into three samples resulted in the following data:

Sample	Amount Added (ng/mL)	Observed (ng/mL)	%Recovery
S1	-	47.984	/
	20.00	67.644	98.30
	100.00	141.934	93.95
S2	-	80.216	/
	20.00	99.266	95.25
	100.00	178.116	97.90
S3	-	161.439	/
	20.00	181.149	98.55
	100.00	262.039	100.60

Method Comparison

A total of 100 clinical samples in the range of 5.02 to 295.70 ng/mL were tested by the T4 assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=1.011x -0.988, r^2=0.991.

Analytical Specificity

The Cross-reactivity of the T4 assay with a cross reactant can be expressed as the ratio of

- The amount of T4 required to displace 50% of the maximally bound labeled T4 from the anti-T4 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	<0.02
Monoiodotyrosine	<0.02
3,5-Diiodo-L-thyronine	<0.02

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 37 mg/dL
- Triglyceride 2500 mg/dL
- Hemoglobin 2300 mg/dL
- Total Protein 12 g/dL
- Rheumatoid factor 620 IU/mL
- HAMA 1232 ng/mL

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