BIO TECHNOLOGY boditech

Hormone

ichroma™ T4

INTENDED USE

ichroma™ T4 is a fluorescence Immunoassay (FIA) for the quantitative determination of thyroxine (T4) in human serum/plasma. It is useful as an aid in management and monitoring of thyroid disorder.

For *in vitro* diagnostic use only.

INTRODUCTION

Thyroxine (T4) is one of two major hormones produced by the thyroid gland (the other is called triiodothyronine, or T3). T4 and T3 are regulated by a sensitive feedback system involving the hypothalamus and the pituitary gland. The hypothalamus releases the thyrotropin-releasing hormone (TRH), which stimulates the pituitary to release the thyroid stimulating hormone (TSH). This causes the thyroid to release T3 and T4 and these in turn regulate the release of TRH and TSH via a feedback control mechanism. Normally, elevated blood levels of T4 and T3 act to decrease the amount of TSH secreted, thereby reducing the production and release of T4 and T3. Over 99 % of T4 is reversibly bound to three plasma proteins in blood: thyroxine binding globulin (TBG) binds close to 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%. Approximately 0.03 % of T4 is in the free, unbound state in blood at any one time.

T4 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T4 decreases in hypothyroidism, myxedema and chronic thyroiditis (Hashimoto's disease). Increased levels of T4 have been found in hyperthyroidism due to Grave's disease and Plummer's disease.

PRINCIPLE

The test uses a competitive immunodetection method.
In this method, the target material in the sample binds to the fluorescence (FL)-labeled detection antibody in detection buffer, to form the complex as sample mixture. This complex is loaded to migrate onto the nitrocellulose matrix, where the covalent couple of T4 and bovine serum albumin (BSA) is immobilized on a test strip, and interferes with the binding of target material and FL-labeled antibody. If the more target material exists in blood, the less detection antibody is accumulated, resulting in the less fluorescence signal.

COMPONENTS

ichroma™ T4 consists of 'cartridges', 'Solution A Tubes', 'Solution B Vial' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has bovine serum albumin (BSA) conjugated T4 at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The solution A pre-dispensed in a tube contains ANS and sodium azide, NaOH in phosphate buffered saline.
- The solution B is dispensed in a vial contains anti human T4-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline.
- The solution A, B are packaged together in a single box. The box will be placed in a Styrofoam box with ice pack for shipping.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and solution A & B) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A solution A tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, solution A, solution B and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ T4** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used solution A, B, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ T4** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ T4** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **sodium heparin, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The solution A pre-dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- The solution B dispensed in a vial is stable for 20 months if stored at 2-8 °C.
- Opened Solution B is stable for 12 months at 2-8 °C if kept in the capped original container and free from contaminations.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician

including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-26

Components of **ichroma™ T4**

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
- Box containing SolutionA, B
  - Solution A tubes 25
  - Solution B Vial (2.5 mL) 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ T4**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ D** REF 13303
- **ichroma™ Printer** REF FPRR007
- **Boditech Hormone Control** REF CFPO-95

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ T4** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The serum or plasma should be separated from the clot by centrifugation within 3 hours after the collection of whole blood. If longer storage is required, e.g. if the test could not be performed within 24 hours, serum or plasma should be immediately frozen below -20 °C. The freezing storage of sample up to 3 months does not affect the quality of results.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of **ichroma™ T4**: Sealed Cartridge, Solution A tube, Solution B Vial and ID Chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the solution A & B.
- Keep the sealed cartridge (if stored in refrigerator), solution A and solution B at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ tests.
- Insert the ID Chip into the ID chip port of the instrument for ichroma™ tests**.**
- Press the 'Select' button on the instrument for ichroma™ tests. (Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)

CAUTION

- To minimize erroneous test results, we suggest that the ambient temperature of the cartridge should be 25 °C during the reaction time after loading sample mixture to the cartridge.
- To maintain the ambient temperature to 25 °C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

1) Transfer 75 µL of sample (Human serum/plasma/control) using a transfer pipette to a tube containing the solution A (yellow tube).
2) Mix well by pipetting 10 times.
3) Add 75 µL of solution B using a transfer pipette with new tip to the tube containing the solution A and sample mixture.
4) Close the lid of the solution A tube and mix the sample thoroughly by shaking it about 10 times.
5) Incubate the solution A + Solution B + sample mixture at room temperature for 8 minutes.
6) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
7) Insert the sample-loaded test cartridge into the slot of the i-Chamber or an incubator (25 ℃).
8) Leave the sample-loaded test cartridge in the i-Chamber or an incubator for 8 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
9) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
10) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
11) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
12) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays T4 concentration of the test sample in terms of nmol/L and µg/dL.
- T4 Conversion factor is 12.87 (nmol/L = 12.87 X µg/dL)
- The cut-off (reference range)

State	Range
Normal value	57.9-150.6 nmol/L

- Working range : 10.23-300.0 nmol/L

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ T4**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank	(LoB)	6.87 nmol/L
Limit of Detection	(LoD)	9.39 nmol/L
Limit of Quantification	(LoQ)	10.23 nmol/L

- **Analytical specificity**

- **Cross-reactivity**

There was no significant cross-reactivity from these materials with the **ichroma™ T4** test measurements.

Compound (Spiked concentration)	Cross-reactivity (%)
l-Triiodothyronine	3.1
reverse T3	3.5

l-Thyrosine	0.8
d-Thyrosine	1.2
3-Iodo-L-tyrosine	1.5
salicylic acid	ND

*ND: Not detected

- **Interference**

There was no significant interference from these materials with the **ichroma™ T4** test measurements.

Compound (Spiked concentration)	Interference (%)
D-glucose (60 mM/L)	3.7
L-Ascorbic acid (0.2 mM/L)	3.9
Bilirubin (0.4 mM/L)	3.5
Hemoglobin (2 g/L)	2.7
Cholesterol (13 mM/L)	8.8
Triglyceride (10 mg/mL)	3.6

- **Precision**

- Intra assay

The intra-assay precision was calculated by one evaluator, who tested different concentration of control standard ten times each with three different lots of **ichroma™ T4.**

T4 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)
50	49.61	49.38	53.00	50.66	2.92	5.8
100	108.43	104.04	103.93	105.47	4.63	4.4
150	154.90	155.28	151.44	153.87	6.76	4.4

- Inter-assay

The inter-assay precision was confirmed by 2 different evaluators for 5 days with 3 different lots, testing three times each different concentrations.

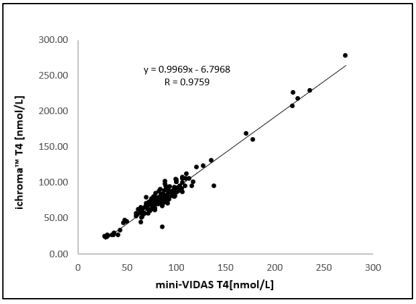
T4 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)
50	51.04	49.09	48.91	49.68	3.01	6.0
100	106.08	108.15	102.17	105.47	5.14	4.9
150	154.18	157.46	151.97	154.54	6.61	4.3

- **Accuracy**

The accuracy was confirmed by 3 different lots testing six times each different concentrations.

T4 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)
12.5	11.19	10.90	11.24	11.11	0.51	4.6
62.5	60.41	63.12	61.01	61.83	2.17	3.5
87.5	81.27	86.04	81.67	82.97	4.96	6.0
125	110.00	122.39	119.54	120.81	6.32	5.2
225	222.19	215.73	214.65	214.82	10.35	4.8

- **Comparability:** T4 concentrations of 143 serum samples were quantified independently with **ichroma™ T4** and mini VIDAS (BioMerieux Inc. France) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y= 0.9969X – 6.7968 and R = 0.9759 respectively.



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Note: Please refer to the table below to identify various symbols

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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