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 anlyte in the sample binds to the fluorescence labeled (FL) detection antibody in detector, 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, and interferes with the binding of analyte and fluorescence labeled (FL) antibody. If more analytes exist in the sample, less detection antibodies are accumulated, resulting in less fluorescence signal.

COMPONENTS

ichroma™ T4 consists of 'Cartridges', 'Detector tube', 'Detector diluent', 'ID chip'and 'Instruction for use'.

- The cartridge contains the membrane called a test strip which has T4-BSA conjugate at the test line, and streptavidin at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant in a box.
- The detector tube has a granule containing anti T4-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in PBS. All detector tubes are packed in a pouch.
- The detector diluent contains 8-anilinonaphthalene-1-sulfonic acid (ANS), Bovine serum albumin, Tween 20 as a detergent, and sodium azide as a preservative in PBS. and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- 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, Detector tube, Detector diluent and ID chip) 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 incorrect test result(s).
- Do not reuse cartridges or detector tube. The detector tube should be used for processing one sample only. A cartridge should be for testing one sample only
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with t local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- Allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent and pipette tips 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.
- No Biotin interference was observed in **ichroma™ T4** when biotin concentration in the sample was below (2 0 ng/mL). If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ T4** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ T4** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant sample.

Recommended anticoagulant
Sodium citrate, Sodium heparin

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	4 - 30 °C.	20 months	Disposable
Detector tube	4 - 30 °C.	20 months	Disposable
Detector diluent	4- 30 °C.	20 months	Unopened
	4- 30 °C.	3 months	Opened

- 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(s) due to the non-responsiveness of the antigen to the antibodies which is most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result 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:
  - Cartridge 25
  - ID chip 1
  - Instruction for Use 1
  - Detector tube 25
  - Detector diluent 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
- **Printer** REF FPRR007
- **i-Chamber** REF FPRR009
- **Boditech Hormone Control** REF CFPO-95
- **Boditech T4 Control** REF CFPO-237

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 Cartridges, Detector tubes, Detector diluent, ID chip and an Instruction for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn 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 200 µL of detector diluent using a pipette to a detector tube containing granule. When the granule is completely dissolved in the detector tube, it becomes detection buffer.
   (The detection buffer must be used immediately within 3 minute right after dissolving the granule.)
2) Transfer 75 µL of sample (Human serum/plasma/ control) using a transfer pipette to a detector tube containing detection buffer.
3) Mix well by pipetting 10 times.
4) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
5) Incubate the detection buffer + 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 °C).

8) Leave the sample-loaded 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 inaccurate 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 is marked on the cartridge especially for this purpose.
10) Press 'Select' or Tap 'START' button on the instrument for ichroma™ tests to start the scanning process.
11) The 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)
- 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)	7.08 nmol/L
Limit of Detection	(LoD)	8.20 nmol/L
Limit of Quantification	(LoQ)	10.23 nmol/L

- **Analytical specificity**

- **Cross-reactivity**
  Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ T4** test results did not show any significant cross-reactivity with these biomolecules.

Compound	Concentration
l-Triiodothyronine	500ng/ml
reverse T3	500ng/ml
l-Thyrosine	300ng/ml
d-Thyrosine	300ng/ml
3-Iodo-L-tyrosine	500ng/ml
salicylic acid	1000,000ng/ml

- **Interference**
  Interference materials such as below the ones in the table were added to the test sample(s) the same as the below concentrations. **ichroma™ T4** test results did not show any significant interference with these materials.

Compound	Concentration
D-glucose	60 mM/L
L-Ascorbic acid	0.2 mM/L
Bilirubin	0.4 mM/L
Hemoglobin	2 g/L
Cholesterol	13 mM/L
Triglyceride	10 mg/ml

- **Precision**
  3 Lots of **ichroma™ T4** were tested for 21days (7days per 1 Lot at 1 site by one operator). Each standard material was tested 2 times per day. For each test, each material was duplicated.

- Repeatability (within-run precision)
  Repeatability of **ichroma™ T4** was evaluated with results of 1 Lot.

- Total precision (within-laboratory precision)
  Total precision(within-run, between-run, between-day) of **ichroma™ T4** are calculated with results of 1 Lot.

T4 [nmol/L]	Repeatability		Total precision (within-laboratory precision)		lot to lot precision	
	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
50	49.90	6.05	50.21	6.2	50.31	6.00
100	99.58	6.95	99.91	6.5	100.52	6.31
150	149.73	6.94	150.01	6.7	150.01	6.32

- Between Site
  Three persons tested **ichroma™ T4** at three different sites, ten times at each concentration of standard materials.

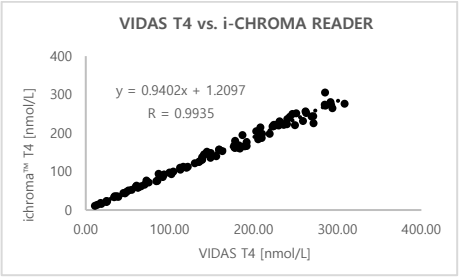
- Between person
  Three persons tested **ichroma™ T4**, ten times at each concentration of standard materials

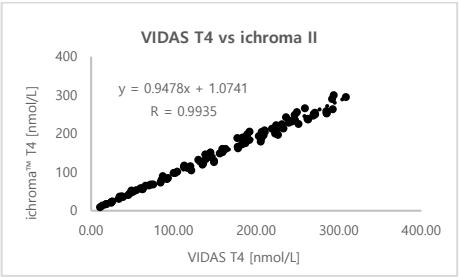
T4 [nmol/L]	Between site		Between person	
	AVG	CV(%)	AVG	CV(%)
50	49.45	3.08	50.21	2.63
100	101.30	6.31	99.32	6.24
150	148.74	9.43	149.75	10.18

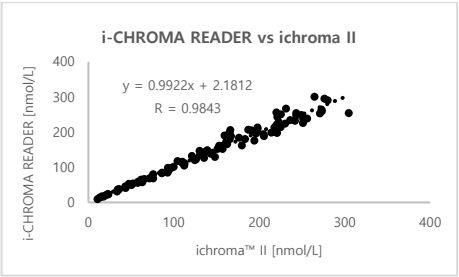
- **Accuracy**
  The accuracy was confirmed by 3 different lots testing ten times each different concentrations.

T4 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery(%)
150	149.35	146.51	148.30	148.05	98.702
75	74.32	74.40	71.90	73.54	98.1
50	47.36	48.77	47.66	47.93	95.9
25	25.29	24.38	24.23	24.63	98.5
12.5	12.33	11.84	12.11	12.09	96.7

- **Comparability**
  T4 concentrations of 100 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 as follows.







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

Σ	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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