Hormone

ichroma™ Free T4

INTENDED USE

ichroma™ Free T4 is a fluorescence immunoassay (FIA) for the quantitative determination of free T4 (free thyroxine) in human whole blood/serum/plasma. It is useful as an aid measurement the assessment of thyroid function.

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.

Free thyroxine acts directly on peripheral tissues as deiodinase within cells to form T3 and binding to the nucleus to indicate the action of hormones. Unlike total thyroxine, it is not affected by TBG, and with some exceptions, if there is an abnormality in free thyroxine, it can be considered as thyroid dysfunction, so the thyroid function.

PRINCIPLE

The test uses a competitive immunodetection method.

The antigen in the sample binds to the fluorescence-labeled detector antibodies in buffer, forming the complexes as a sample mixture. They will migrate onto nitrocellulose matrix, which will interfere with the binding of the free fluorescence-labeled detector antibodies to the immobilized antigens on the test strip.

More antigens in the sample will result in less free detection antibodies to accumulate, which lead to less fluorescence signal by the free fluorescence-labeled detector antibodies. This signal is processed by the instrument for ichroma™ tests to show Free T4 concentration in the sample.

COMPONENTS

ichroma™ Free T4 consists of 'cartridges', 'detector tubes', and 'detector diluent'.

- The cartridge contains the membrane called a test strip, which has streptavidin at the test line and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has 2 granules containing anti-T4-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, T3-biotin conjugate and sodium azide as a preservative in Tris-HCl. All detector tubes are packed in a pouch.
- The detector diluent contains sodium azide as a preservative in Tris-HCl 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 instructions and procedures described in this 'Instructions 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 tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing of 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 local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, the allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent, capillary tubes and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **ichroma™ Free T4** when biotin concentration in the sample was below 5 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™ Free T4** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Free T4** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium heparin

- The capillary tube should be used when the following conditions are met.
  - The capillary tube provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Excess whole blood around the capillary tube should be wiped off.
  - In order to avoid cross-contamination, please do not re-use capillary tube for multiple samples.

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 antigens to the antibodies which is the 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 antigens with time and/or temperature may also cause false negative result as it makes antigens 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 in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30°C	20 months	Disposable
Detector tube	2 - 30°C	20 months	Disposable
Detector diluent	2- 30°C	20 months	Unopened
		20 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-164

Components of **ichroma™ Free T4**

- Cartridge Box:
  - Cartridge 25
  - Detector tube 25
  - 30 µL capillary tube 25
  - Detector diluent 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ Free T4**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
  - **ichroma™-50 PLUS** REF FPRR036
- **i-Chamber** REF FPRR009
- **Boditech Free T4 Control** REF CFPO-375

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Free T4** human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a week at 2-8°C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20°C.
- The samples (serum, plasma) stored frozen at -20°C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Whole blood sample may be used to collect according to below:

① Wear disposable gloves and protective equipment for safety.

② Open the zipper bag which has capillary tubes.

③ Take out the capillary tube and check for damage or contamination.

④ Hold the handle of the capillary tube and touch the surface of blood with the capillary tube.

⑤ Fill it with blood completely.
(Make sure that no air bubbles are present in the capillary tube. Do not get blood on the surface of the capillary tube. If the blood gets on the surface of the capillary tube, remove it gently with gauze.)

TEST SETUP

- Check the contents of **ichroma™ Free T4**: Sealed cartridges, 30 µL capillary tubes, detector tubes, a detector diluent, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as the ID chip.
- If the sealed cartridge, the detector tube and the detector diluent have been stored in 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.
- Insert the ID chip into the 'ID chip port'.

※ **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

▶ **ichroma™ II, ichroma™ M3**

1) Take 150 µL of detector diluent using a pipette and dispense it to the detector tube containing 2 granules. When the granule form is completely dissolved in the detector tube, it becomes detection buffer.
   (The detection buffer must be used immediately. Do not exceed 3 minutes.)
2) Take 30 µL of sample (whole blood/serum/plasma /control) using a pipette and dispense it to the detector tube.
   ※ If the test use whole blood, transfer the blood (collected in a capillary tube) to a detector tube.
3) Mix well by pipetting 10 times and close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
   (The sample mixture must be used immediately. Do not exceed 30 seconds.)
4) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
5) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (25 °C).
6) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 12 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
7) 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.
8) Tab the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
   (ichroma™ M3 will start the test automatically after inserting.)
9) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
10) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ **ichroma™ III**

1) The test procedure is same with 'ichroma™ II test procedure'.

▶ **ichroma™-50 PLUS**

1) Insert the tip array in the tip station.
2) Insert the detector tube in the reagent station and cover the reagent station to hold the detector tube.
3) Open the lid of the detector diluent and insert the detector diluent in the diluent station.
4) Insert the cartridge magazine with the cartridges into the magazine station.
5) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
6) Tap the button located in the upper side of the No. of test cartridge region to select the ID chip that you want to use.
7) When the selected cartridge slot is activated, set the number of the detector tube by tapping.
8) Set the number of pipette tips by tapping.
9) Tap the 'Start' button on the left upper of the main screen to start test.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays Free T4 concentration of the test sample in terms of pmol/L.
- Working range : 1-100 pmol/L
- Reference range: 9 – 22 pmol/L
- Conversion factor: 12.87 (SI: pmol/L = 12.87 x ng/dL)

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.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ Free T4**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 0.53 pmol/L
  - Limit of Detection (LoD) 0.87 pmol/L
  - Limit of Quantitation (LoQ) 1.00 pmol/L
- **Hook effect**
  No high-dose effect is observed at L-Thyroxine concentrations up to 1,200 pmol/L.
- **Analytical specificity**
  - Cross reactivity
    Biomolecules listed in the following table were added to the test samples at concentrations much higher than their normal physiological levels in the blood. **ichroma™ Free T4** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactivity materials	Concentration
l-Triiodothyronine	500 ng/mL
reverse T3	500 ng/mL
L-Tyrosine	300 ng/mL

D-Tyrosine	300 ng/mL
3-iodo-L-tyrosine	500 ng/mL

- Interference

Interferents listed in the following table were added to the test samples at the concentration mentioned below. **ichroma™ Free T4** test results did not show any significant interference with these materials except for Cholesterol. **ichroma™ Free T4** does not recommend the use of lipid-rich samples.

Interferents	Concentration
D-glucose	60 mM/L
L-Ascorbic acid	0.3 mM/L
Bilirubin(unconjugated)	0.7 mM/L
Hemoglobin	1000 mg/dL
Triglyceride	50 g/L
salicylic acid	1,000 ug/mL
Cholesterol	13 mM/L

■ **Precision**

- Single-site study

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **ichroma™ Free T4** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
Free T4 [pmol/L]	**Repeatability**		**Within-laboratory precision**		**Lot to lot precision**	
	AVG [pmol/L]	CV (%)	AVG [pmol/L]	CV (%)	AVG [pmol/L]	CV (%)
6.25	6.09	15.50	6.08	14.96	6.19	15.17
12.50	12.51	14.65	12.19	14.23	12.31	14.09
50.00	49.69	9.04	49.83	8.94	49.85	8.46

- Multi-site study

Reproducibility

1 Lot of **ichroma™ Free T4** was tested for 5 days in 3 Different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study		
Reproducibility		
Free T4 [pmol/L]	AVG [pmol/L]	CV (%)
6.25	6.04	15.05
12.50	12.41	14.85
50.00	50.35	7.62

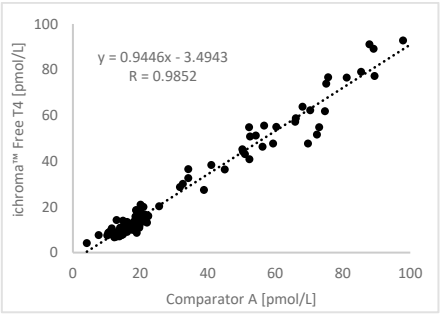
■ **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ Free T4.** The tests are repeated 10 times in each different concentration.

Free T4 [pmol/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
50.00	53.72	53.78	53.83	53.78	107.6
41.25	39.98	43.66	43.68	42.44	102.9
32.50	37.09	34.43	34.19	35.24	108.4
23.75	21.91	22.82	21.02	21.91	92.3
15.00	15.72	15.36	14.87	15.32	102.1
6.25	6.70	6.07	6.78	6.52	104.3

■ **Comparability**

Free T4 concentration of 100 clinical samples were quantified independently with **ichroma™ Free T4 (ichroma™ II)** and comparator A as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follow.



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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
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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