Hormone

ichroma™ T3

INTENDED USE

ichroma™ T3 is a fluorescence immunoassay (FIA) for the quantitative determination of total T3 (total triiodothyronine) in human serum/plasma. It is useful as an aid in management and monitoring of determination of thyroid disorders.

For *in vitro* diagnostic use only.

INTRODUCTION

3,5,3' Triiodothyronine (T3) is a thyroid hormone with a molecular weight of 651 daltons.[1]

T3 circulates in the blood as an equilibrium mixture of free and protein bound hormone.[2] T3 is bound to thyroxin binding globulin (TBG), prealbumin, and albumin. The actual distribution of T3 among these binding proteins is controversial as estimates range from 38-80% for TBG, 9-27% for prealbumin, and 11-35% for albumin.[3]

T3 plays an important role in the maintenance of the euthyroid state. T3 measurements can be a valuable component in diagnosing certain disorders of thyroid function.[4] Most reports indicate that T3 levels distinguish clearly between euthyroid and hyperthyroid subjects, but provide a less clear-cut separation between hypothyroid and euthyroid subjects.[5] Total T3 measurements may be valuable when hyperthyroidism is suspected and the free T4 is normal.[6] For example, one recognized type of thyroid dysfunction is T3 thyrotoxicosis, associated with a decrease in serum thyroid stimulating hormone (TSH), increased T3 level, normal T4, normal free T4, and normal to increase in vitro Uptake results.[7-11]

T3 levels are affected by conditions which affect TBG concentration.[12-14] Slightly elevated T3 levels may occur in pregnancy or during estrogen therapy, while depressed levels may occur during severe illness, renal failure, myocardial infarction, alcoholism, inadequate nutritional intake, and during therapy with some medications such as dopamine, glucocorticoids, methimazole, propranolol, propylthiouracil, and salicylates.[6,15,16]

Numerous conditions unrelated to thyroid disease may cause abnormal T3 values.[5, 17-20] Consequently, total T3 values should not be used on their own in establishing the thyroid status of an individual. The level of serum T4, TSH and other clinical findings must be considered as well.

PRINCIPLE

The test uses a competitive immunodetection method.

The antigens in the sample bind 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 T3 concentration in the sample.

COMPONENTS

ichroma™ T3 consists of 'cartridges', 'detector tubes' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has T3-BSA conjugate 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 a granule containing anti-T3-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate and sodium azide as a preservative in sodium phosphate buffer. All detector tubes are packed in a pouch.
- The detector diluent contains 8-anilinonaphthalene-1-sulfonic acid (ANS) and Tween 20 as detergent, sodium azide as a preservative in sodium hydroxide solution. It is pre-dispensed in 2 vials. The detector diluents are packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the 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 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, then 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 diluents 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™ T3** when biotin concentration in the sample was below 3,500 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 take.
- **ichroma™ T3** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ T3** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium heparin

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 the 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
		3 months	Opened

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

MATERIALS SUPPLIED

REF CFPC-44

Components of **ichroma™ T3**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 2
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ T3**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M3** REF FPRR035
- **Printer** REF FPRR007
- **i-Chamber** REF FPRR009
- **Boditech T3 Control** REF CFPO-240
- **Boditech Hormone Control** REF CFPO-95

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ T3** is human 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 (serum, plasma) may be stored for a month at 2-8°C prior to being tested. If testing will be delayed more than a month, samples (serum, plasma) should be frozen at -20°C.
- The samples (serum, plasma) stored frozen at -20°C for 2 months showed no performance difference.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ T3**: Sealed cartridges, detector tubes, detector diluents, 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 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.
- 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

▶ **ichorma™ Reader, ichorma™ II, ichroma™ M3**

1) Take 300 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
   (The detection buffer must be used immediately. Do not exceed 30 seconds.)
2) Take 75 µL of sample (serum/plasma/control) using a pipette and dispense it to the detector tube. Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
   (The detection buffer must be used immediately. Do not exceed 30 seconds.)
3) Incubate the sample and the detection buffer mixture at room temperature for 8 minutes.
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 8 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) Press the 'Select' or tap 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.

▶ **ichorma™ III**

1) The test procedure is same with 'ichroma™ Reader, ichroma™ II, ichroma™ M3 1) – 4)'.
2) Insert the sample-loaded cartridge into the holder of the ichroma™ III. 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.
3) Tap the 'Start' button on the ichroma™ III to start the scanning process.
4) The cartridge goes inside and ichroma™ III will automatically start scanning the sample-loaded cartridge after 8 minutes.
5) Read the test result on the display screen of the ichroma™ III.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays T3 concentration of the test sample in terms of ng/mL and nmol/L.
- Reference range

State	N	[ng/mL]	[nmol/L] (SI unit)
Normal	59	0.8 ~ 2.01	1.24 ~ 3.09

- Working range: 0.5-5.0 ng/mL (0.77-7.7 nmol/L)
- Conversion factor as unit of nmol/L
  - nmol/L (SI unit) = 1.54 × ng/mL
  - ng/dl = 100 × ng/mL

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™ T3**. 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)	0.23 nmol/L
Limit of Detection (LoD)	0.45 nmol/L
Limit of Quantitation (LoQ)	0.77 nmol/L

- **Hook effect**
  No high-dose effect was observed in this assay at T3 concentrations up to 46.2 nmol/L.

- **Analytical specificity**
  - Cross reactivity
    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ T3** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
D-thyroxine	300 ng/mL
L-thyroxine	300 ng/mL
Reverse T3	500 ng/mL
Salicylic acid	1,000,000 ng/mL
Monoiodotyrosine	50,000 ng/mL

  - Interference
    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ T3** test results did not show any significant interference with these materials except for K_2 EDTA, sodium citrate and Cholesterol.
  - K_2 EDTA, sodium citrate as an anticoagulant are not recommended with **ichroma™ T3**.
  - The use of lipid-rich sample is not recommended for **ichroma™ T3**.

Interferents	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
K_2 EDTA	10.8 mg/mL
Sodium Heparin	54 mg/mL
Sodium Citrate	40 mg/mL

- **Precision**

One person tested three standard materials (three lot every 7 days) twice a day (Run, morning/afternoon) and twice repeated (duplicate) in the same place for 21 days.

- Repeatability (within-run precision)
  To evaluate repeatability, the mean value and CV (%) were calculated from the results of Run 1 in Lot 1.
- Total precision (within-laboratory precision)
  To evaluate total precision, the mean value and CV (%) are calculated from all the results of Lot 1.
- Lot to lot precision
  Lot to lot precision was evaluated from the results of 3 lots.

T3 [nmol/L]	Repeatability		Total precision		Lot to lot precision	
	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)
1.08	1.09	6.63	1.08	6.9	1.08	6.77
2.31	2.32	6.26	2.31	6.6	2.32	6.25
6.16	6.16	6.58	6.17	6.3	6.18	6.22

- Between site
  Three persons tested **ichroma™ T3** at three different sites, ten times at each concentration of standard materials.
- Between person
  Three persons tested **ichroma™ T3**, ten times at each concentration of standard materials.

T3 [nmol/L]	Between-site		Between-person	
	AVG [nmol/L]	CV (%)	AVG [nmol/L]	CV (%)
1.08	1.08	5.85	1.08	6.24
2.31	2.27	6.34	2.32	4.86
6.12	6.16	5.62	6.14	6.32

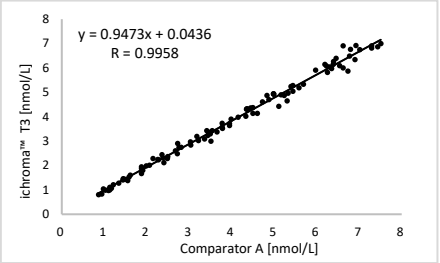
- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ T3**. The tests were repeated 10 times at each concentration of the control standard.

T3 [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
6.16	6.05	6.14	6.09	6.09	98.91
5.14	5.11	5.27	5.33	5.23	101.8
4.13	4.14	4.09	4.24	4.15	100.7
3.11	3.18	3.16	3.05	3.13	100.7
2.09	2.08	2.05	2.09	2.07	99.0
1.08	1.09	1.12	1.04	1.08	100.5

- **Comparability**

T3 concentrations of 100 clinical samples were quantified independently with **ichroma™ T3 (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 follows.



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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

For technical assistance, please contact:
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