Hormone

ichroma™ Testosterone

INTENDED USE

ichroma™ Testosterone is a fluorescence immunoassay (FIA) for the quantitative determination of Testosterone in human serum/plasma. It is useful as an aid in management and monitoring of androgen level.

For *in vitro* diagnostic use only.

INTRODUCTION

Testosterone (17ß-hydroxyandrost-4-en-3-one) is an anabolic steroid synthesized primarily by Leydig cells in the testes of male, the ovary of female, and adrenal glands of both sexes[1]. It is synthesized from cholesterol, androstenediol, Dehydroepiandrosterone (DHEA), progesterone, and pregnenolone acting as some of the intermediate substrates. Testosterone level in male increase 10 to 20-fold during puberty, driving the physiological changes associated with male puberty. It also exerts a powerful, wide-ranging influence over emotional well-being, sexual function, muscle mass and strength, energy, cardiovascular health, bone integrity, and cognitive ability throughout a man's entire life. In the blood only 1 to 15% of testosterone is in its unbound or biologically active form. The remaining testosterone is bound to serum proteins.

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-antigen 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 testosterone concentration in the sample.

COMPONENTS

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

- The cartridge contains the membrane called a test strip which has Testosterone-BSA conjugate at the test line, Rabbit IgG at the control line and anti-mouse IgG at the antigen 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-rabbit IgG fluorescence conjugate, anti-testosterone-fluorescence conjugate, and sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a pouch.
- The detector diluent contains bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline and they are 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 '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 cartridge 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 a refrigerator, then allow cartridge 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, sample mixing tubes and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and detector diluent contain sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and 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.
- **ichroma™ Testosterone** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Testosterone** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2EDTA

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 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 antigen with time and/or temperature may also cause false negative result as it makes the 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 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
	2 - 30°C	3 months	Opened

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

MATERIALS SUPPLIED

REF 13012

Components of **ichroma™ Testosterone**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Sample mixing 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™ Testosterone**.

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 Hormone Control** REF CFPO-95
- **Boditech Testosterone Control** REF CFPO-239

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Testosterone** 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 week at 2-8°C prior to being tested. If the 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 2 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.

TEST SETUP

- Check the contents of **ichroma™ Testosterone**: Sealed cartridges, 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 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

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

1) Take 60 µL of detector diluent using a pipette and dispense it to the sample mixing tube.
2) Take 150 µL of sample (serum/plasma/control) using a pipette and dispense it to the sample mixing tube. Close the lid of the sample mixing tube and mix the sample thoroughly by shaking it about 10 times.
   Incubate the tube at room temperature for 3 minutes.
3) Take 150 µL of sample mixture using a pipette and dispense it to the detector tube containing a granule. Close the lid of the detector tube and mix the sample thoroughly by shaking 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) 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.

▶ **ichroma™ III**

1) The test procedure is same with the 'ichroma™ II test procedure 1) ~ 4)'.
2) Insert the sample-loaded cartridge into the holder of 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 ichroma™ III to start the scanning process.
~~4)~~ The cartridge goes inside and ichroma™ III will automatically start scanning the sample-loaded cartridge after 12 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 testosterone concentration of the test sample in terms of ng/mL.
- Working range: 0.5-12 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™ Testosterone**. 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) 0.188 ng/mL
- Limit of Detection (LoD) 0.295 ng/mL
- Limit of Quantitation (LoQ) 0.500 ng/mL

■ **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™ Testosterone** test result did not show any significant cross-reactivity with these biomolecules.

Cross reactants	Concentration
Androstenedione	1,000 ng/ml
Androsterone	100,000 ng/ml
Cortisol	8,000 ng/ml
Estradiol	1,000 ng/ml
Danazol	1,000 ng/ml
5-a-DHT	50 ng/ml
DHEA	10,000 ng/ml
Oxymetholone	100 ng/ml
Estrone	500 ng/ml
Corticosterone	5,000 ng/ml
Methyltestosterone	100 ng/ml
11-Deoxycortisol	1,000 ng/ml
Progesterone	1,000 ng/ml
19-Nor Testo	1,000 ng/ml

- Interference

Interferents listed in the following table were added to the test sample(s) the concentrations below. **ichroma™ Testosterone** test result did not show any significant interference with these materials.

Interferents	Concentration
Bilirubin [unconjugated]	40 mg/dL
Triglycerides	1,500 mg/dL
Albumin	5,200 mg/dL
Ascorbic acid	1,000 mg/dL

■ **Precision**

- **Single-site study**

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

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

Single-site study						
Testosterone [ng/mL]	Repeatability		within-laboratory precision		Lot to lot precision	
	AVG [ng/mL]	CV (%)	AVG [ng/mL]	CV (%)	AVG [ng/mL]	CV (%)
3	3.06	7.8	3.05	8.4	3.03	7.9
6	6.04	5.9	6.06	5.9	6.05	6.2
9	9.06	5.8	8.99	5.9	8.97	6.1

- **Multi-site study**

Reproducibility

1 Lot of **ichroma™ Testosterone** 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	
Testosterone [ng/mL]	Reproducibility
	AVG [ng/mL]	CV (%)
3	3.03	7.5
6	6.05	5.8
9	9.10	5.8

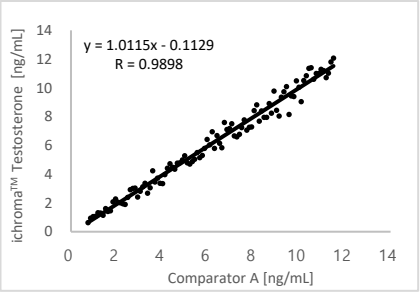
■ **Accuracy**

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

Testosterone [ng/mL]	Lot 1	Lot 2	Lot 3	AVG [ng/mL]	Recovery (%)
12.00	12.20	11.57	12.53	12.10	101
9.70	9.69	9.57	9.39	9.55	98
7.40	7.40	7.38	7.56	7.44	101
5.10	5.13	5.20	5.06	5.13	101
2.80	2.77	2.86	2.79	2.81	100
0.50	0.50	0.49	0.52	0.50	101

■ **Comparability**

Testosterone concentration of 100 clinical samples were quantified independently with **ichroma™ Testosterone** (**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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2. Vining, R.F., and McGinley, R.A. The measurement of hormones in saliva: Possibilities and pitfalls. Journal of Steroid Biochemistry, 1987, 27: 81-94.
3. Tulsidas G. Shrivastav. Matrix interference in direct total Testosterone enzyme immunoassay and It's elimination with the use of non-cross reactivity steroids in serum based standards. Health and Population Perspectives and Issues, 2002,25(2):55-64.

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

For technical assistance, please contact:
Boditech Med Inc.'s Technical Services
Tel: +(82) -33-243-1400
E-mail: TS@boditech.co.kr

Boditech Med Inc.
43, Geodudanji 1-gil, Dongnae-myeon, Chuncheon-si, Gang-won-do, 24398, Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

EC REP **Obelis s.a**
Bd. Général Wahis 53, 1030 Brussels, Belgium
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

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