BIO TECHNOLOGY boditech

Hormone

ichroma™ LH

INTENDED USE

ichroma™ LH is a fluorescence Immunoassay (FIA) for the quantitative determination of Luteinizing hormone (LH) in human serum/plasma. It is useful as an aid in management and monitoring of determination of evaluating fertility issues, function of reproductive organs (ovaries or testicles), or detection of the ovulation.

For *in vitro* diagnostic use only.

INTRODUCTION

Human luteinizing hormone (LH, lutropin) is a glycoprotein hormone with two dissimilar subunits (α and β). LH has a molecular weight of approximately 29,000 daltons.[1] The α-subunit of LH contains 92 amino acid residues and is essentially identical to the β-subunits of follicle stimulating hormone (FSH, follitropin), thyroid stimulating hormone (TSH, thyrotropin), and human chorionic gonadotropin (hCG).[1-4] The β-subunit of LH contains 112 amino acid residues and is considerably different from that of FSH and TSH.[1,4,5] However, the β-subunits of LH and hCG are very similar. The structural similarities between LH and hCG are responsible for the observed similarity in biological properties.[1,5,6] In the female, hLH stimulates the final maturation of the follicle, follicular rupture, and ovulation.[7] Human LH is secreted by the gonadotropic cells of the anterior lobe of the pituitary gland in response to gonadotropin releasing hormone (GnRH) from the medial basal hypothalamus. Both hLH and hFSH are secreted in a pulsatile nature; however, this is less noticeable for hFSH perhaps due to the longer half-life in the circulation.[7] In a normal menstrual cycle negative feedback by estradiol suppresses hLH secretion in the follicular phase. As the follicle develops (in response to hFSH) estradiol production increases which triggers an increase in GnRH and an increased sensitivity of the pituitary to GnRH. A GnRH surge results in the preovulatory (mid-cycle) surge of hLH and ovulation. Following this surge, hLH is suppressed during the luteal phase due to negative feedback from progesterone and estradiol.[7-9] Variation in cycle lengths are observed in normally menstruating females due to variations in the length of the follicular phase. In the menopausal female, hLH levels are elevated in response to decreased production of ovarian estrogens and progestogens, which eliminates the negative feedback mechanism on the pituitary gland. As a result, ovulation and menstrual cycles decrease and eventually cease.[10] In the male, hLH is often referred to as interstitial cell-stimulating hormone and influences the production of testosterone by the Leydig cells of the testes.[11] At menopause, or following ovariectomy in women, concentrations of estrogens decline to low levels. The lowered concentrations of estrogens result in a loss of the negative feedback on gonadotropin release. The consequence is an increase in the concentrations of LH and FSH.[12,13,14] Concentrations of hLH and hFSH are commonly determined in investigations of menstrual cycle, fertility, and pubertal developmental abnormalities, such as premature ovarian failure, menopause, ovulatory disorders and pituitary failure.[15] The ratio of hLH/hFSH has been used to assist in the diagnosis of polycystic ovary disease. Low concentrations of hLH and hFSH may indicate pituitary failure while elevated concentrations of hLH and hFSH along with decreased concentrations of gonadal steroids may indicate gonadal failure (menopause, ovariectomy, premature ovarian syndrome, Turners Syndrome).[16] Low concentrations of gonadotropin are usually observed in females taking oral steroid based contraceptives.[17] In the male, elevated hLH and hFSH with low concentrations of gonadal steroids may indicate testicular failure or anorchia. In Klinefelter's syndrome hLH may be elevated due to Sertoli cell failure.[18]

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in the sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by instrument for ichroma™ tests to show LH concentration in the sample.

COMPONENTS

ichroma™ LH consists of 'cartridges', and 'detection buffer tubes'.

- The cartridge contains the membrane called a test strip which has anti human LH at the test line, while rabbit IgG 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 detection buffer contains anti human LH-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in CAPSO buffer.
- The detection buffer is pre-dispensed in tubes. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

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, ID chip and detection buffer) 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 cartridges of detection buffer tubes. A cartridge should be used for testing one sample only. A detection buffer 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 already 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 be used.
- Allow cartridge, detection buffer 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, detection buffer 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.
- **ichroma™ LH** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ LH** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than EDTA, sodium heparin, sodium citrate should be avoided.

STORAGE AND STABILITY

Storage condition		
Component	Storage Temperature	Shelf life
Cartridge	4 - 30 °C	20 months
Detection buffer tube	2 - 8 °C	20 months

- 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 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 in conjunction with clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF 13010

Components of **ichroma™ LH**

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction for Use 1
- Buffer Box
  - Detection buffer tube 25

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from ichroma™ LH.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
- **Printer** REF FPRR007
- **Boditech LH Control** REF CFPO-234
- **Boditech Hormone Control** REF CFPO-95

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ LH** 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.
- Samples may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C.
- Samples 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™ LH**: Sealed Cartridges, Detection buffer tubes, ID Chip and Instruction for use.
- Ensure that the lot number of the cartridges matches that of the detection buffer tube as well as ID chip.
- If the sealed cartridge and the detection buffer 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.)

TEST PROCEDURE

1) Transfer 150 µL (Human serum/plasma/control) of sample using a pipette to a tube containing the detection buffer.
2) Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately.)
3) Pipette out 75 µL of a sample mixture and load it into the sample well on the cartridge.
4) Leave the sample-loaded cartridge at room temperature for 15 minutes.
⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
5) 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.
6) Press 'Select' or tap 'START' button on the instrument for ichroma™ tests to start the scanning process.
7) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
8) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

■ The instrument for ichroma™ tests calculates the test result automatically and displays LH concentration of the test sample in terms of mIU/mL.
■ Cut-off (reference value):

Type		mIU/mL
Males		1.79 – 7.68
Females	Follicular phase	1.48 – 12.40
	Ovulatory phase	16.47 – 73.87
	Luteal phase	0.64 – 14.67
Postmenopausal		11.49 – 40.62

■ Working range : 1.0-100.0 mIU/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.
■ 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™ LH**. 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)	0.29 mIU/mL
Limit of Detection (LoD)	0.4 mIU/mL
Limit of Quantification (LoQ)	1 mIU/mL

■ **Analytical specificity**

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

Cross reactivity materials	Concentration of cross reactivity materials	Cross reactivity (%)
hCG	15,000 mIU/mL	0.55
FSH	1,500 mIU/mL	N/D
PRL	1,500 mIU/mL	N/D
TSH	1,500 mIU/mL	0.31

* ND : Not Detected

- **Interference:**
Study of interference from table below with ichroma™ LH showed following results.

Interference materials	Concentration of interference materials	Interference (%)
D-glucose	60 mM/L	< 1.3
L-Ascorbic acid	0.2 mM/L	< 2.6
Bilirubin [unconjugated]	0.4 mM/L	< 2.8
Hemoglobin[human]	2 g/L	< 3.0
Cholesterol	13 mM/L	< 4.5
Triglyceride	10 mg/mL	< 3.1
Sodium citrate	16mg/mL	< 3.5
Sodium heparine	100U/mL	< 3.4
EDTA	7.5mg/mL	< 2.3

■ **Precision**

- Between Lot
One person tested three different lots of **ichroma™ LH**, ten times at each concentration of the control standard.
- Between person
Three different persons tested same lot of **ichroma™ LH**, ten times at each concentration of the control standard.
- Between day
One person tested same lot of **ichroma™ LH**, during five days, five times at each concentration of the control standard.
- Between site
Three different persons tested same lot of **ichroma™ LH** at three different sites, five times at each concentration of the control standard.

LH	Between-lot		Between-person		Between-day	
(mIU/mL)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)
5.00	5.30	3.4	5.32	3.07	5.38	5.1
10.00	10.19	7.3	10.17	5.65	10.19	7.1
50.00	53.22	5.0	52.71	5.39	52.31	4.4

LH	Between-site	
(mIU/mL)	AVG	CV(%)
5.00	5.37	5.0
10.00	10.29	7.1
50.00	52.51	4.2

■ **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ LH**.

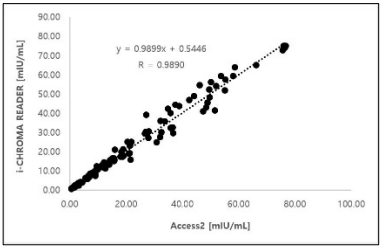
The tests are repeated ten times in each different concentration.

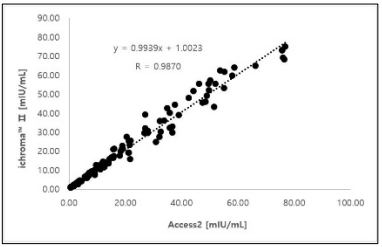
LH (mIU/mL)	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
3.0	2.87	3.01	2.96	2.95	98%
7.5	7.42	7.59	7.45	7.49	100%
25.5	25.80	25.63	24.56	25.33	99%
55.0	58.91	54.49	54.44	55.95	102%
75.0	74.10	76.34	73.46	74.63	100%

■ **Comparability**

LH concentrations of 119 serum samples were quantified independently with **ichroma™ LH** (i-CHROMA READER, ichroma™ II) and Access2 (Beckman Coulter Inc. USA) 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 are as follows respectively.

	Access 2	
	Linear regresstion	Coefficient of correlation (R)
i-CHROMA READER	Y= 0.9899X + 0.5446	R=0.9890
Ichroma™ II	Y= 0.9939X + 0.1023	R=0.9870





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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

For technical assistance; please contact:
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CE IVD