Hormone

ichroma™ LH

INTENDED USE

ichroma™ LH is a fluorescence immunoassay (FIA) for the quantitative determination of LH (Luteinizing hormone) 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. 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). The β-subunit of LH contains 112 amino acid residues and is considerably different from that of FSH and TSH. 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. In the female, hLH stimulates the final maturation of the follicle, follicular rupture, and ovulation. 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. 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. 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. 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. 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. 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. 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). Low concentrations of gonadotropin are usually observed in females taking oral steroid based contraceptives. 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.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized antibodies on a 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 the instrument for ichroma™ tests to show LH concentration in the sample.

COMPONENTS

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

- The cartridge contains the membrane called a test strip which has anti-LH at the test line, and rabbit IgG at the control line. All the 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-LH fluorescence conjugate, anti-rabbit IgG-fluorescence conjugate and sodium azide as a preservative in potassium phosphate buffer (KPI). All detector tubes are packed in a pouch.
- The detector diluent contains tween-20 as a detergent and sodium azide as a preservative in CAPSO buffer, 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 '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, 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 diluent 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 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.
- **ichroma™ LH** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ LH** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, 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 the 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 13010

Components of **ichroma™ LH**

- Cartridge Box:
  - Cartridge 25
  - Detector 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™ LH**.

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
- **Boditech Hormone Control** REF CFPO-95
- **Boditech LH Control** REF CFPO-234

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 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 room temperature or 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℃ 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.

TEST SETUP

■ Check the contents of **ichroma™ LH**: 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.**

TEST PROCEDURE

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

Multi test mode

1) Take 150 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 150 µL of sample (serum/plasma/control) using a pipette and dispense it to the detector tube.
3) 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) Leave the cartridge at room temperature for 15 minutes.
⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
6) 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.
7) 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.)
8) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode

1) The test procedure is same with the 'Multi test mode 1) – 4)'.
2) Insert the sample-loaded cartridge into the 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.
3) Press the 'Select' or tap the 'Start' button on the instrument for ichroma™ tests.
(ichroma™ M3 will start the test automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 15 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ **ichroma™ III**

1) The test procedure is same with the 'Single test mode'.

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.
■ Reference value

Type		mIU/mL
Males		1.24–8.62
Females	Follicular phase	2.12–10.89
	Mid-cycle	19.18–103.03
	Luteal phase	1.20–12.86
	Postmenopausal	10.87–58.64

■ 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.
■ 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™ LH**. 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) 0.292 mIU/mL
- Limit of Detection (LoD) 0.526 mIU/mL
- Limit of Quantitation (LoQ) 1 mIU/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™ LH** test result did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
FSH	2,000 mIU/mL
hCG	500,000 mIU/mL
PRL	2,000 ng/mL
TSH	2,000 μIU/mL

- Interference

Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ LH** test result did not show any significant interference with these materials.

Interferents	Concentration
D-glucose	1000 mg/dL
Albumin (Protein)	60 g/L
L-Ascorbic acid	300 μmol/L
Bilirubin[unconjugated]	684 μmol/L
Hemoglobin(human)	200 g/L
triglyceride	2500 mg/mL
Acetaminophen	1030 μmol/L
Ibuprofen	1060 μmol/L
Acetylsalicylic acid	167 μmol/L
Caffeine	556 μmol/L
Heparin	330 units/dL
EDTA	3.39 μmol/L

■ **Precision**

- Single-site study

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

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

Single-site study						
LH [mIU/mL]	Repeatability		Within-laboratory precision		Lot to lot precision	
	AVG [mIU/mL]	CV (%)	AVG [mIU/mL]	CV (%)	AVG [mIU/mL]	CV (%)
5	5.00	5.0	5.01	5.5	5.02	5.4
10	10.02	5.7	9.99	6.1	9.96	6.0
50	50.08	5.6	50.09	5.4	50.14	5.9

- Multi-site study

Reproducibility

1 Lot of **ichroma™ LH** 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		
LH [mIU/mL]	Reproducibility	
	AVG [mIU/mL]	CV (%)
5	5.02	5.7
10	10.07	5.4
50	49.31	6.1

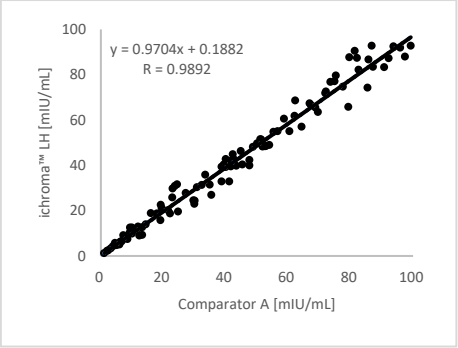
■ **Accuracy**

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

LH [mIU/mL]	Lot 1	Lot 2	Lot 3	AVG [mIU/mL]	Recovery
1	1.05	1.06	1.06	1.06	105.5%
2	2.02	1.94	1.98	1.98	99.1%
3	2.98	2.92	3.06	2.99	99.5%
4	4.01	3.93	4.13	4.03	100.6%
5	5.01	4.92	5.07	5.00	100.0%
10	9.77	10.23	9.95	9.98	99.8%
20	19.83	20.28	19.43	19.84	99.2%
50	51.10	52.65	49.59	51.11	102.2%
100	96.60	97.00	94.26	95.96	96.0%

■ **Comparability**

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



REFERENCES

1. Pierce JG, Parsons TF. Glycoprotein Hormones: Structure and Function. *Annu Rev Biochem* 1981; 50:465-95.
2. Shome B, Parlow AF. Human Follicle Stimulating Hormone (hFSH): First Proposal For the Amino Acid Sequence of the α-Subunit and First Demonstration of its Identity with the α- Subunit of Human Luteinizing Hormone (hLHα) *J Clin Endocrinol Metab* 1974; 39:199-202.
3. Sairam MR, Li CH. Human Pituitary Thyrotropin: Isolation and Chemical Characterization of its Subunits. *Biochem Biophys Res Commun* 1973; 51:336-42.
4. Vaitukaitis JL, Ross GT, Braunstein GD, *et al.* Gonadotropins and Their Subunits: Basic and Clinical Studies. *Recent Prog Horm Res* 1976; 32:289-331.
5. Bishop WH, Nureddin A, Ryan RJ. Pituitary Luteinizing and Follicle-Stimulating Hormones. In: Parsons JA, editor. Peptide Hormones. Baltimore: University Park Press, 1976:273-98.
6. Keutmann HT, Williams RM, Ryan RJ. Structure of

Human Luteinizing Hormone Beta Subunit: Evidence for a Related Carboxyl-Terminal Sequence Among Certain Peptide Hormones. *Biochem Biophys Res Commun* 1979; 90:842-8.

7. South SA, Yankov VI, Evans WS. Normal reproductive neuroendocrinology in the female. In Endocrinology and Metabolism Clinics of North America 1993; Edited by Veldhuis JD, Philadelphia, PA: W.B. Saunders Co. 22: 1-28.
8. Adashi EY. The ovarian life cycle. In Reproductive Endocrinology. Edited by Yen, S.S.C., Jaffe, R.B., Philadelphia, W.B. Saunders Co. 1991; 181-237.
9. Yen SSC. The human menstrual cycle: neuroendocrine regulation. In Reproductive Endocrinology 1991; Edited by Yen, S.S.C., Jaffe, RB, Philadelphia, PA: W.B. Saunders Co., 273-308.
10. Richardson SJ. The biological basis of menopause. Baillières Clinical Endocrinology and Metabolism 1993; 7:1-16.
11. Reyes-Fuentes A, Veldhuis JD. Neuroendocrine physiology of the normal male gonadal axis. In Endocrinology and Metabolism Clinics of North America 1993; Edited by Veldhuis, JD. Philadelphia, PA: W.B. Saunders Co., 22: 93-124.
12. Ross GT. Disorders of the Ovary and Female Reproductive Tract. In: Wilson JD and Foster DW, editors. Williams Textbook of Endocrinology. Philadelphia: Saunders, 1985:206-58.
13. Beastall GH, *et al*. Assays for Follicle Stimulating Hormone and Luteinizing Hormone: Guidelines for the Provision of a Clinical Biochemistry Service. *Ann Clin Biochem* 1987; 24:246-62.
14. Judd HL. Hormonal Dynamics Associated with the Menopause. *Clin Obstet Gynecol* 1976; 19:775-88.
15. Carr BR, Disorders of the ovary and female reproductive tract. In Williams Textbook of Endocrinology 8th edition 1992; Edited by Wilson JD and Foster DW, Philadelphia, PA: W. B. Saunders Co., 733-798.
16. Hall JE. Polycystic ovarian disease as a neuroendocrine disorder of the female reproductive axis. In Endocrinology and Metabolism Clinics of North America 1993; Edited by Veldhuis JD, Philadelphia, PA: W.B. Saunders Co. 22 (1): 75-92.
17. Bonnar J. The hypothalmus and reproductive function. In The Medical Annual 1973; Edited by Scott RB and Walker RM, Bristol, England, J. Wright and Sons, 251-258.
18. Tietz Textbook of Clinical Chemistry. 1994. Second edition. Edited by Burtis CA and Ashwood ER, Philadelphia, PA: W. B. Saunders Co. 1846-1850.
19. National Institutes of Health. Historical reference range of Luteinizing Hormone. http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index/A166B3FACB4F69C285256BA4006B37ED?OpenDocument.

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

	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

CE IVD