Hormone

ichroma™ Progesterone

INTENDED USE

ichroma™ Progesterone is a fluorescence immunoassay (FIA) for the quantitative determination of progesterone in human serum/plasma. It is useful as an aid in management and monitoring of the cause of infertility, track ovulation, diagnose an ectopic or failing pregnancy, monitor the health of a pregnancy.

For *in vitro* diagnostic use only.

INTRODUCTION

Progesterone also known as P4 (pregn-4-ene-3,20-dione) is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy (supports gestation) and embryogenesis of humans and other species.[2] Progesterone belongs to a class of hormones called progestogens, and is the major naturally occurring human progestogen.

In mammals, progesterone, like all other steroid hormones, is synthesized from pregnenolone, which in turn is derived from cholesterol.

Progesterone is essential for the regulation of normal female reproductive functions. The major physiological actions of progesterone are: a) in the uterus and ovary: induction of ovulation, facilitation of implantation, and maintenance of early pregnancy; b) in the mammary gland: lobular-alveolar development in preparation for milk secretion[3,4]; c) in the brain: neurobehavioral expression associated with sexual responsiveness[5] and d) in the bone: prevention of bone loss[6].

During the follicular phase of the cycle, progesterone levels remain low[7-9]. Following the LH surge and ovulation, luteal cells in the ruptured follicle produce progesterone in response to LH. During this, the luteal phase, progesterone rises rapidly to a maximum of 10-20 ng/mL at day 5-7 following ovulation. During the luteal phase, progesterone transforms the estrogen-primed endometrium from a proliferative to a secretory state.[8] If pregnancy does not occur, progesterone levels decrease during the last four days of the cycle due to the regression of the corpus luteum.[7,8-13] If conception occurs, the levels of progesterone are maintained at mid-luteal levels by the corpus luteum until about week six. At that time the placenta becomes the main source of progesterone and levels rise from approximately 10-50 ng/mL in the first trimester to approximately 50-280 ng/mL in the third trimester.[7,14,15]

PRINCIPLE

The test uses a competitive immunodetection method. The analyte 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, where the covalent couple of progesterone and bovine serum albumin (BSA) is immobilized and interferes with the binding of analyte and fluorescence labeled antibody. If more analytes exist in the sample, less detection antibodies are accumulated, resulting in less fluorescence signal.

COMPONENTS

ichroma™ Progesterone consists of 'cartridges', detector tubes', 'detector diluent'.

- The cartridge contains the membrane called a test strip which BSA-Progesterone conjugate at the test line, and streptavidin 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 human progesterone-fluorescence conjugate, biotin – BSA – fluorescence conjugate, bovine serum albumin (BSA), sucrose as a stabilizer and sodium azide as a preservative in phosphate buffered saline. All detector tubes are packed in a pouch.
- The detector diluent contains bovine serum albumin (BSA) as a stabilizer, sodium azide as a preservative, and Tween20 as a detergent in phosphate buffered saline, 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 for testing one sample only. The detector tube should be used for processing 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.
- If test components and/or sample are stored in refrigerator, the allow the 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 the 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.

- No Biotin interference was observed in **ichroma™ Progesterone** when biotin concentration in the sample was below 100 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™ Progesterone** will provide accurate and reliable results subject to the below conditions.

- **ichroma™ Progesterone** 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

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.

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 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.

MATERIALS SUPPLIED

REF CFPC-21

Components of **ichroma™ Progesterone**

- Cartridge Box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - ID chip 1
  - Instruction for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

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

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
  - **ichroma™-50 PLUS** REF FPRR036
- **Printer** REF FPRR007
- **i-chamber** REF FPRR009
- **Boditech Hormone Control** REF CFPO-95
- **Boditech Progesterone Control** REF CFPO-238

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Progesterone** is human serum/plasma.

- To avoid time related absorption, serum samples should not be stored in collection tube with gel separators.
- It is recommended to test the sample within 24 hours after collection.
- 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 testing will be delayed more than a week, samples should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 6 month 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™ Progesterone**: Sealed cartridges, detector tubes, (a) detector diluent(s), an ID chip and 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 test cartridge should be 25°C during the reaction time after loading sample

mixture to the test 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 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 30 µL of sample (Human 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 sample mixture must be used immediately. Do not exceed 30 seconds.)
3) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
4) Insert the sample-loaded cartridge into the slot of the i-chamber or an incubator (25 °C).
5) Leave the sample-loaded cartridge in the i-chamber or an incubator 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 'Select' button or tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
   (ichroma™ M3 is tested 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.

▶ **ichroma™ III**

1) The test procedure is same with 'Multi test mode 1) – 3)'.
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 the ichroma™ III.
4) The cartridge goes inside and ichroma™ III will automatically start scanning the sample-loaded cartridge after 15 minutes.
5) Read the test result on the display screen of ichroma™ III.

▶ **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 tubes in place.
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 progesterone concentration of the test sample in terms of nmol/L and ng/mL.
- Reference range

Reference Group	Mean (ng/mL)
Male	0.84
Females	
mid-follicular phase	0.69
mid-luteal phase	11.42
post menopausal	0.25
Pregnancy	
first trimester	22.17
second	29.73

* SI: 1 ng/mL = 3.18 nmol/L

- Working range: 4.45-127.2 nmol/L and 1.4-40 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™ Progesterone**. 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)	1.52 nmol/L
Limit of Detection (LoD)	2.336 nmol/L
Limit of Quantitation (LoQ)	4.45 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™ Progesterone** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactivity materials	Concentration of cross reactivity materials
17-α-OH-progesterone	2 μg/mL
17β-estradiol(estradiol)	2 μg/mL
5 α-prognane-3, 20-dione	0.2 μg/mL
Hydrocortisone	2 μg/mL
Danazol	20 μg/mL
Estriol	2 μg/mL
Testosterone	2 μg/mL
Dexamethasone	2 μg/mL
Estrone	2 μg/mL
Transferrin	2 μg/mL

*N/D: Not Detection

- Interference

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

Interference materials	Concentration of interference materials
D-glucose	600 mM
L-Ascorbic acid	2 mM
Bilirubin [unconjugated]	4 mM
Hemoglobin[human]	20 g/L
Cholesterol	130 mM
Triglyceride	100 mg/mL

■ **Precision**

- Repeatability (within-run precision)

1 Lot of **ichroma™ Progesterone** was tested for 21 days. 3 concentrations of standard materials were tested 2 times per day with 2 replicates respectively. Within 2 runs of tests every day, run 1 was used to verify the repeatability of **ichroma™ Progesterone.**

- Total precision (within-laboratory precision)

With those test results for the repeatability analysis of **ichroma™ Progesterone**, the entire test result was used to verify the total precision of **ichroma™ Progesterone**.

- Lot to lot precision

3 Lots of **ichroma™ Progesterone** were tested for 21 days with standard materials at 3 different concentrations 2 times per day with 2 replicates respectively to demonstrate lot to lot precision of **ichroma™ Progesterone**.

- Between-person

3 different operators performed test using 3 different lots with standard materials at 3 different concentrations 10 times respectively to verify between-site precision of **ichroma™ Progesterone**.

- Between-site

At three different sites, the standard at 3 different concentrations were tested 10 times with 3 different lots to verify Between site precision of **ichroma™ Progesterone**.

- Between-reader

3 different operators performed the test with the standard materials at 3 different concentrations with 3 analyzers (ichroma™ Reader) 10 times respectively to verify between-reader precision.

Conc. (nmol/L)	Repeatability (within-run)		Total precision (within-laboratory precision)	
	AVG	CV(%)	AVG	CV(%)
12.72	12.67	8.8	12.73	9.0
31.8	32.43	7.6	32.13	7.4
63.6	62.71	8.2	63.05	8.2
Conc.	Lot to lot precision		Between-person	
(nmol/L)	AVG	CV(%)	AVG	CV (%)
12.72	12.75	8.8	12.69	8.4
31.8	31.95	8.1	31.68	8.8
63.6	63.76	8.7	63.59	8.8
Conc.	Between-site		Between-reader	
(nmol/L)	AVG	CV (%)	AVG	CV (%)
12.72	12.95	7.5	12.56	8.9
31.8	31.76	7.8	31.81	8.0
63.6	62.88	8.8	62.04	8.1

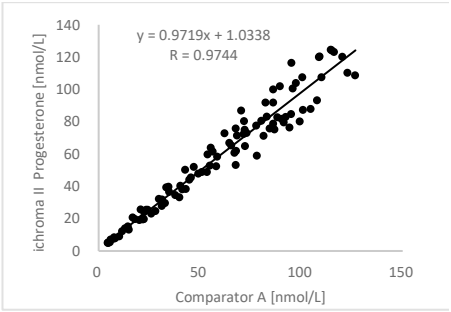
■ **Accuracy**

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

Expected value [nmol/L]	Lot 4	Lot 5	Lot 6	AVG	Recovery (%)
5.1	4.82	4.82	4.98	4.87	95.5%
8.0	7.91	7.88	7.37	7.72	96.5%
11.6	11.19	11.83	11.52	11.51	99.3%
21.2	21.37	22.46	21.62	21.82	102.9%
42.4	40.51	40.19	40.46	40.38	95.2%
50.9	47.24	48.17	47.79	47.73	93.8%
63.6	66.26	68.64	65.25	66.71	104.9%
76.3	76.69	71.48	77.58	75.25	98.6%
95.4	97.96	92.73	89.72	93.47	98.0%

■ **Comparability**

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



REFERENCES

1. Potential use of single measurement of serum progesterone in detecting early pregnancy failure Hanita O MD, MPATH, Hanisah AH MD, MPATH
2. Metabocard for Hydroxyprogesterone. Human Metabolome Database. Retrieved 31 July 2013.
3. Progestin regulation of cellular proliferation. Clark CL and Sutherland RL. Endocrine Review 1990;11: 266-301.
4. Physiological Action of Progesterone in Target Tissues. Graham JD and Clarke CL. Endocrine Reviews 1997;18: 502-519.
5. Progesterone, progestagens and the central nervous system. Hum Reprod Genazzani AR, Stomati M, Morittu A, Bernardi F, Monteleone P, Casarosa E, Gallo R, Salvestroni C and Luisi M. 2000; 15: 14-27.
6. Sex steroids and bone: current perspectives. Hum reprod update. Balasch J. 2003; 9: 207-22.
7. Simultaneous Radioimmunoassay of Plasma FSH, LH, Progesterone, 17-Hydroxyprogesterone, and Estradiol-17 beta During the Menstrual Cycle. Abraham GE, Odell WD, Swerdloff RS, Hopper K. J Clin Endocrinol Metab, 1972; 34:2, 312–318.
8. Studies on the Pattern of Circulating Steroids in the Normal Menstrual Cycle. Aedo AR, Nunez M, Landgren BM, Cekan SZ, Diczfalusy E. Circadian Variation in Theperi-Ovulatory Period. Acta Endocrinol (Copenh), 1977; 84:2, 320-332
9. Hormonal Profile of the Cycle in 68 Normally Menstruating Women. Landgren BM, Unden AL, and Diczfalusy E. Acta Endocrinol (Copenh), 1980; 94:1, 89-98.
10. Normal Ovarian Function. Erickson GG. Clin Obstet Gynecol, 1978; vol. 21 No.1, 31-53.
11. Physiological Profiles of Episodic Progesterone Release During the Midluteal Phase of the Human Menstrual Cycle: Analysis of Circadian and Ultradian Rhythms, Discrete Pulse Properties, and Correlations with Simultaneous Luteinizing Hormone Release. Veldhuis JD, Christiansen E, Evans WS, Kolp LA, Rogol AD, Johnson ML. J Clin Endocrinol Metab, 1988; 66:2, 414-421.
12. Neuroendocrine Regulation of the Corpus Luteum in the Human. Evidence for Pulsatile Progesterone Secretion. Filicori M, Butler JP, Crowley WF Jr., J Clin Invest, 1984; 73:6, 1638-1647.
13. The Pattern of Luteal Phase Plasma Progesterone and Estradiol in Fertile Cycles. Laufer N, Navot D, Schenker JG. Am J Obstet Gynecol, 1982; 143:7, 808-813.
14. Method for Monitoring Plasma Progesterone Concentrations in Pregnancy. Winkel P, Gaede P, Lyngbye J Clin Chem 1976; 22:4, 422-428.
15. The Applications of Steroid Hormone Radioimmunoassays to Clinical Obstetrics. Buster JE, Abraham GE. Obstet Gynecol, 1975; 46:4, 489-499.

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

CE IVD