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. In this method, the analyte in the sample binds to the fluorescence labeled (FL) detection antibody in detection buffer, to form the complex as sample mixture. This complex is loaded to migrate onto the 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 (FL) 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 tube', 'Detector diluent', 'ID chip' and 'Instruction for Use'.

- 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 in a box.
- The detector tube has a granule containing anti human progesterone-fluorescence conjugate, biotin – BSA – fluorescence conjugate, bovine serum albumin (BSA) 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 sodium azide as a preservative in distilled water, 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) 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 tube. 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. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- Allow the cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used detector tubes, pipette tips and cartridges 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™ Progesterone** will provide accurate and reliable results subject to the below conditions.
- **ichroma™ Progesterone** should be used only in conjunction with instrument for ichroma™ tests.
- Have to use recommended anticoagulant sample.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, sodium heparin

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	4 - 30 °C	20 months	Disposable
Detector tube	4 - 30 °C	20 months	Disposable
Detector diluent	4- 30 °C	20 months	Unopened
	4- 30 °C	20 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 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 including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-21
Components of **ichroma™ Progesterone**

- Cartridge Box:
  - Cartridge 25
  - ID chip 1
  - Instruction for use 1
  - Detector tube 25
  - Detector diluent 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
- **Printer** REF FPRR007
- **i-Chamber** REF FPRR009
- **Boditech Hormone Control** REF CFPO-95

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 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 up to 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.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the contents of **ichroma™ Progesterone**: Sealed cartridges, detector tubes, detector diluent, ID chip and Instruction for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, detector diluent as well as the 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.
  (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

1) Transfer 150 µL of detector diluent using a pipette to a detector tube containing granule. When the granule is completely dissolved in the detector tube, it becomes detection buffer.
   (The detection buffer must be used immediately within 30 seconds.)
2) Transfer 30 µL of sample (Human serum/plasma/ control) using a transfer pipette 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 within 30 seconds right after shaking 10 times.)
4) Pipette out 75 µL of a sample mixture and load it into the sample well on 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 15 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 'Select' button or Tap 'START' button on the instrument for ichroma™ tests to start the scanning process.
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.

INTERPRETATION OF TEST RESULT

- 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

Type		Mean (nmol/L)	Range (nmol/L)
Males		2.67	0.46-6.55
Non-pregnant Females	Mid-follicular phase	2.19	0.99-4.83
	Mid-luteal phase	36.32	16.4-59.02
	Post-menopausal	0.8	<0.25-2.48
Pregnancy	First trimester	70.5	15.04-161.35
	Second trimester	94.54	61.72-144.05

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

- 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.
- 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™ Progesterone**. 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)	1.2 nmol/L
Limit of Detection (LoD)	1.7 nmol/L
Limit of Quantification (LoQ)	4.45 nmol/L

- **Analytical specificity**

- Cross-reactivity
  There was no significant cross-reactivity from these materials with the **ichroma™ Progesterone** test measurements.

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
  There was no significant interference from these materials with the **ichroma™ Progesterone** test measurements.

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

■ **Precision**

- **Repeatability / Total precision / Lot to Lot precision**
  3 Lots of **ichroma™ Progesterone** were tested for 21 days (7 days per 1 Lot). Three concentrations of standard materials were tested 2 times per day. For each test, each material was duplicated.
- **Repeatability (within-run precision)**
  Repeatability was evaluated by using the results from run 1 of Lot 1 tests in the between day study.
- **Total precision (within-Laboratory precision)**
  Total precision was evaluated by using the total test result from Lot 1.
- **Lot to Lot precision**
  Lot to Lot precision was evaluated by using 3 different lot.
- **Between person**
  Three different persons tested **ichroma™ Progesterone**; ten times at each concentration of the control standard.
- **Between site**
  One person tested **ichroma™ Progesterone** at three different sites; ten times at each concentration of the control standard.

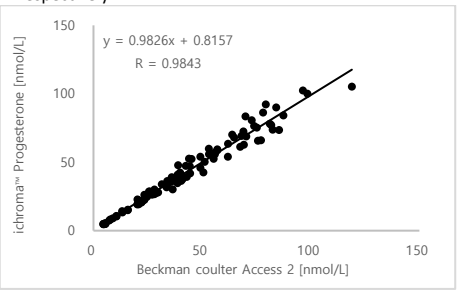
Conc. (nmol/L)	Repeatability (within-run)		Total precision (within-laboratory precision)	
	AVG	CV(%)	AVG	CV(%)
12.72	12.92	5.5	12.81	5.4
31.8	31.36	5.2	31.55	5.4
63.6	63.21	6.6	63.14	6.2
Conc. (nmol/L)	Lot to lot precision		Between-person	
	AVG	CV(%)	AVG	CV (%)
12.72	12.73	5.64	12.66	5.23
31.8	31.86	5.55	32	5.92
63.6	63.42	5.88	62.9	5.8

Conc. (nmol/L)	Between-site	
	AVG	CV (%)
12.72	12.64	5.63
31.8	31.74	5.45
63.6	63.4	6.02

■ **Accuracy**
The accuracy was confirmed by 3 different lots testing ten times each different concentration.

Conc. (nmol/L)	Lot 1	Lot 2	Lot 3	AVG	Recovery
4.6	4.5	4.6	4.6	4.6	100%
7.6	7.68	7.68	7.41	7.59	99%
11.4	11.62	11.34	11.33	11.43	100%
22.9	23.17	22.52	22.69	22.79	100%
38.2	38.43	38.05	37.8	38.09	100%
47.7	47.78	46.73	47.32	47.28	99%
68.7	71.45	68.31	69.62	69.79	102%
76.3	76.95	76.53	76.53	76.67	100%
95.4	95.77	93.21	96.94	95.31	100%

■ **Comparability**
Progesterone concentrations of 100 clinical samples were quantified independently with **ichroma™ Progesterone** and Beckman Coulter Access2 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 were Y=0.9826X + 0.8157 and R = 0.9843 respectively.



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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:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr


Boditech Med Incorporated
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


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