Hormone

ichroma™ Estradiol

INTENDED USE

ichroma™ Estradiol is a fluorescence immunoassay (FIA) for the quantitative determination of Estradiol in human whole blood/serum/plasma. It is useful as an aid in management and monitoring of Estrogen abnormalities.

For *in vitro* diagnostic use.

INTRODUCTION

Estradiol (17-β estradiol) is the primary ovarian hormone involved in the development of the female reproductive system. It is a form of estrogen that regulates various physiological functions, including ovulation and the development of female secondary sex characteristics. Estradiol is classically considered a reproductive hormone due to its role in feedback signaling within the hypothalamic-pituitary-ovarian axis.[1,2,3]

The concentration of estradiol remains relatively low in men, as well as in women before puberty and after menopause. However, it increases significantly during puberty in women.[3,4]

Estradiol, along with estrone (E1) and estriol (E3), belongs to the group of sex steroids known as estrogens. Among these, estradiol is the most biologically active and predominant form.[5]

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 streptavidin 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 Estradiol concentration in the sample.

COMPONENTS

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

- The cartridge contains the membrane called a test strip which has streptavidin at test line, and chicken IgY 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 2 granules containing anti-Estradiol-fluorescence conjugate, anti-Estradiol-biotin conjugate, anti-chicken IgY-fluorescence conjugate and sodium azide as a preservative. All detector tubes are packed in a pouch.
- The detector diluent contains detergent and sodium azide as a preservative in phosphate buffered saline. 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 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 the 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.
- No Biotin interference was observed in **ichroma™ Estradiol** when biotin concentration in the sample was below 200 ng/mL. If a patient has been taking biotin at dosage of more than 20 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ Estradiol** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Estradiol** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Li-heparin

LIMITATIONS 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 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 CFPC-190

Components of **ichroma™ Estradiol**

- 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™ Estradiol**.

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M2** REF FPRR031
- **Boditech Estradiol Control** REF CFPO-434

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Estradiol** is human whole blood/serum/plasma.

- It is recommended to test the whole blood sample immediately after collection when collected sample is stored at room temperature (15 - 25°C). If testing will be delayed, whole blood can be stored at 2 - 8°C for up to 3 hours before testing.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- It is recommended to test the serum and plasma samples within 8 hours after collection when collected samples are stored at room temperature (15 - 25°C).
- The samples (serum, plasma) may be stored for 48 hours at 2 - 8°C prior to being tested. If testing will be delayed more than 48 hours, samples should be frozen at -20°C.
- The samples (serum, plasma) stored frozen at -20°C for 2 months showed no performance difference.
- The samples (serum, plasma) stored at -20°C should use only once.
- 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™ Estradiol**: 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 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™ test.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for ichroma™ tests operation manual for complete information and operation instructions.**

TEST PROCEDURE

▶ **ichroma™ II, ichroma™ M2**

Multi test mode (Read now mode)

1) Select the sample type (whole blood or serum/plasma) on the screen.
2) Take 150 µL of the 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.)
3) Take 50 µL of sample (whole blood/serum/plasma/control) using a pipette and dispense it to the detector tube.
4) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
5) Leave the sample mixture at room temperature for 5 minutes.
6) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
7) 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.*
8) 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.

9) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M2 will start the test automatically after inserting.)
10) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
11) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode (Walk away mode)

1) The test procedure is same with 'Multi test 1) – 6)'.
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) Tap the 'Start' button on the instrument for ichroma™ tests.
(ichroma™ M2 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 Estradiol concentration of the test sample in terms of pg/mL.
- Working range: 20 - 3,000 pg/mL
- Conversion factors: pg/mL x 3.67 = pmol/L

Non-pregnant females	Estradiol level [pg/mL]
Early follicular	8.4-209.7 (31-771 pmol/L)
Late follicular	28.3-473.8 (104-1742 pmol/L)
LH Peak	74.8-779.0 (275-2864 pmol/L)
Early luteal	25.8-323.1 (95-1188 pmol/L)
Mid luteal	41.1-481.2 (151-1941 pmol/L)
Late luteal	10.6-481.2 (39-1769 pmol/L)

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™ Estradiol**. 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) 7 pg/mL
  - Limit of Detection (LoD) 20 pg/mL
  - Limit of Quantitation (LoQ) 30 pg/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™ Estradiol** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	concentration
Aldosterone	100 µg/mL
Cortisol	200 µmol/L
17a-Estradiol	0.5 µg/mL
17b-Estradiol 3 glucuronide	0.3 µg/mL
Estradiol valerate	0.5 µg/mL
Estriol	0.5 µg/mL
Estrone	0.3 µg/mL

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

Interferents	concentration
Acetaminophen	20 mg/dL
Ascorbic acid	350 µmol/L
Billiubin	0.7 mM/L
Biotin	200 ng/mL
Hemoglobin	1,000 mg/dL
Triglyceride	15 g/L
Cholesterol	400 mg/dL

- **Precision**

- **Single-site study**
Repeatability (within-run precision)
Within-laboratory precision (Total precision)
Lot to lot precision
3 Lots of **ichroma™ Estradiol** were tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
Estradiol [pg/mL]	Repeatability		within-laboratory precision		Lot to lot precision	
	AVG [pg/mL]	CV (%)	AVG [pg/mL]	CV (%)	AVG [pg/mL]	CV (%)
30.24	28.42	12.4	29.28	13.2	31.01	11.1
298.12	307.94	9.3	301.33	9.8	298.46	7.1
1210.50	1210.35	8.3	1275.12	10.5	1207.91	6.7

- **Multi-site study**
Between persons
3 Lots of **ichroma™ Estradiol** were tested 10 times by 3 persons at each concentration of the standard material.
Between sites
1 Lot of **ichroma™ Estradiol** was tested 10 times at 3 different sites at each concentration of the standard material.

Between readers

1 Lot of **ichroma™ Estradiol** was tested 10 times with 3 different readers at each concentration of the standard material.

Multi-site study						
Estradiol [pg/mL]	Between-persons		Between-site		Between-readers	
	AVG [pg/mL]	CV (%)	AVG [pg/mL]	CV (%)	AVG [pg/mL]	CV (%)
30.24	30.79	11.8	29.39	10.1	29.91	10.5
298.12	310.81	8.6	303.77	7.4	297.11	7.3
1210.50	1264.45	6.2	1237.08	5.2	1209.95	5.8

- **Accuracy**

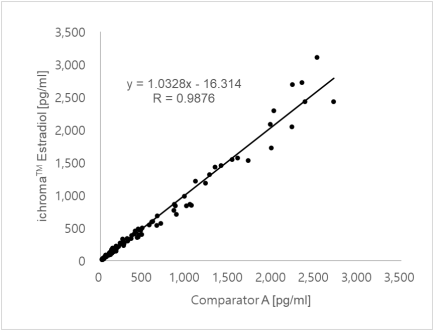
The accuracy was confirmed by testing with 3 different lots of **ichroma™ Estradiol**. The tests were repeated 10 times at each concentration of the control standard

Estradiol [pg/mL]	Lot 1	Lot2	Lot 3	AVG [pg/mL]	Recovery (%)
62.0	67.87	61.76	59.62	63.08	101.7
124.5	123.41	121.10	125.78	123.43	99.1
498.5	507.22	533.48	468.36	503.02	100.9
1002.4	997.99	1051.70	1001.87	1017.19	101.5
1510.4	1597.12	1497.34	1503.14	1532.53	101.5
2008.8	2017.50	1988.77	1964.31	1990.19	99.1

- **Comparability**

Estradiol concentration of 100 clinical samples were quantified independently with **ichroma™ Estradiol (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.

* Solid line: regression line of **ichroma™ Estradiol**



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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
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

For technical assistance, please contact:
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IVD