Instructions for Use

Estradiol (CLIA)

[Product Name]

Estradiol (CLIA)

[Packing Size]

24×1 Tests/ Pkg (Calibrators included); 60×1 Tests/ Pkg (Calibrators included); 60×1 Tests/ Pkg

[Intended Use]

Estradiol (E2) assay is a chemiluminescent immunoassay used for the quantitative determination of estradiol (E2) in human serum and plasma. The results of the assay are mainly used for the auxiliary diagnosis of ovarian diseases.

Estradiol, i.e. 17β-estradiol, is a natural estrogen with a molecular weight of 272.3 Da. Most of the circulating estradiol is bound to sex hormone binding globulin (SHBG) or human serum albumin, and only 1~3% of the total estradiol is free [1, 2]. Estradiol is the most active estrogen in the human body. It is responsible for regulating female reproductive function, and maintains pregnancy together with progesterone. Estradiol is secreted primarily by the ovary and corpus luteum in non-pregnant women. But small quantity of estradiol is also formed in the testes (males) and in the adrenal cortex (males and females) [3]. In pregnant women, most estradiol is produced by the placenta [4, 5]. During menstruation, estradiol level is at the lowest. Then it rises in the late follicle development, and reaches the highest level before LH (luteinizing hormone) surges. Normally, ovulation begins after LH surges. When LH reaches the peak, estradiol level decreases, and then rises again until the luteal phase. If conception does not occur, estradiol will drop further and menstruation will follow. After menopause, estradiol concentration remain low in women [6-8]. The estradiol concentration of female reflects follicular maturity, it can be used to assess ovarian function and to monitor ovulation. Therefore, monitoring of estradiol is important to assess and analyze sexual development status, amenorrhea aetiology, infertility, and menopause [9-12]. Normally, estradiol in men is very low, and abnormally high estradiol level in men is a sign of feminization syndrome [13].

[Principle of the Assay]

E2 immunoassay takes a competition format. The detection principle is described below:

(1) Sample, acridinium-labeled anti-E2 antibody conjugate, and reaction diluent are mixed and incubated. E2 in the sample binds to anti-E2 antibody

(2) The reaction solution is transferred to the well containing E2-derivate coated magnetic microparticle. After mixing and incubation, free acridinium-labeled anti-E2 antibody unbound by E2 in the reaction mixture will bind to the E2-derivative coated microparticle, and forms a complex.

(3) A magnet captures the microparticle, and then unbound substance in solution is washed off. Add pre-trigger and trigger solution to the reaction mixture sequentially to initiate chemiluminescence reaction.

(4) A photomultiplier tube is used to measure photons generated from the reaction. The count is inversely proportional to E2 concentration in the sample. Concentration of E2 is determined by an internal calibration curve.

[Main Components]

Packing Size

Component \ Packing Size	Fill Volume		
	24×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg
E2 Reagent Cartridge – Microparticle (R1)	24×50 μL	60×50 μL	60×50 μL
E2 Reagent Cartridge – Conjugate (R2)	24×100 μL	60×100 μL	60×100 μL
E2 Reagent Cartridge – Reaction diluent (R3)	24×150 μL	60×150 μL	60×150 μL
E2 Calibrator – E2 Calibrator C1	1×1.0 mL	1×1.0 mL	/
E2 Calibrator – E2 Calibrator C2	1×1.0 mL	1×1.0 mL	/
Calibration Card	1 pcs	1 pcs	/

Main Composition

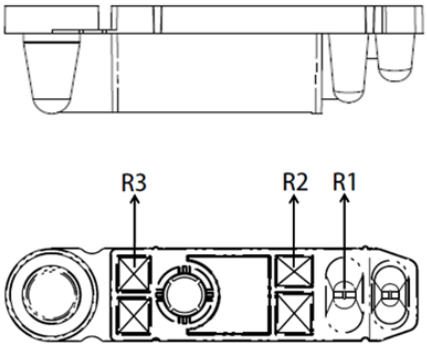
Component		Main Composition
E2 Reagent Cartridge	Microparticle (R1)	E2-derivative coated microparticle, ~0.3 g/L; Tris buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Conjugate (R2)	Acridinium labeled mouse-anti-E2 antibody, ~200 μg/L; MES buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Reaction diluent (R3)	Tris buffer, 50 mmol/L; ProClin 300, 0.5 g/L
E2 Calibrator	E2 Calibrator C1	Estradiol; Tris buffer, 25 mmol/L; ProClin 300, 0.5 g/L
	E2 Calibrator C2	Estradiol; Tris buffer, 25 mmol/L; ProClin 300, 0.5 g/L
Calibration Card		Calibration curve and calibrator information

Note: (1) Components in different lots of reagent cannot be mixed or exchanged for use.

(2) Traceability: This quantification method can be traced back to Roche Elecsys Estradiol III assay.

(3) Information about the calibrators (such as lot number and concentration etc.) can be found on the instrument interface after scanning the Calibrator Card.

The position of each component in the reagent pack is shown in the front view (Upper) and vertical view (Down) of the reagent cartridge.



Instruments and accessories needed but not supplied (Medcaptain has the supplies)

(1) Medcaptain Immu F6/F6S Automatic Chemiluminescent Immunoassay Analyzers;

(2) Pre-trigger solution;

(3) Trigger solution;

(4) Washing solution;

(5) 500 μL pipette tips;

(6) Sexual Hormone Controls.

[Storage and Shelf-life]

Storage: Store sealed reagent cartridge and calibrators at 2~8℃ in upright position,

and avoid freezing.

Shelf life: 14 months.

Stability of Calibrators: Sealed vial of calibrators can be kept at 2~8℃ in dark for 14 months. After calibrator C1 or C2 is opened and sealed again, it is allowed to be stored at 10~30℃ for 5 days, and at 2~8℃ for 60 days.

The dates of manufacturing and expiration can be found on the labels.

[Matched Instruments]

Medcaptain Immu F6/F6S Automatic Chemiluminescent Immunoassay Analyzers

[Specimen Types]

Serum, or plasma (collected with EDTA-K_2, EDTA-K_3, lithium heparin, and sodium heparin as anti-coagulants) can be used in the testing.

Sample volume for each test: 50 μL.

The collected sample should be tested as soon as possible. Serum and plasma can be kept at 10~30℃ for 8 hours, at 2~8℃ for 48 hours, and at -20℃ or below for 60 days. Frequent freeze-thaw cycles should be avoided, and only one freeze-thaw cycle is allowed. If the sample contains precipitate or frozen floccule, centrifugation is needed to clear the sample before testing.

Sample collection tubes from different manufacturers may lead to variation in testing results, due to the difference in tube material and additives in the tubes. Medcaptain has not evaluated all types of sample collection tubes from different manufacturers. Each laboratory should make its own judgment about the usability of sample collection tubes.

[Testing Procedure]

Reagent Preparation

Reagent: E2 reagent cartridge (containing magnetic microparticle R1, acridinium labeled conjugate R2, and reaction diluent R3) is ready for use. It can be loaded directly into the instrument after opening the package.

Calibrators: Calibrator C1 and C2 are ready for use. Each calibrator can be added to a sample cup, and the cups are loaded to a sample rack. The sample rack can be pushed directly into the instrument for calibration tests.

Calibration

Refer to the relevant chapter of system calibration in the operation manual of each chemiluminescent immunoassay analyzer. Calibration tests should be ordered before the first time use of E2 assay. Medcaptain provides E2 reagent pack and matched calibrators to calibrate the instrument.

Before calibration, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put calibrators on a sample rack of the analyzer, and load the rack into the instrument. On the screen interface of “Reagent > Request Calibration”, select test name and lot number to request a calibration. Select the position of each calibrator on the sample rack, set the number of repeat tests, start calibration.

Automatic immunoassay analyzer makes use of calibration data to validate the calibration, and adjust the calibration curve automatically.

Instrument calibration is effective for 60 days. A new calibration is needed in the following situation:

(1) Change into a new lot of reagent;

(2) Control test results are out of the target range;

(3) The lot of reagent has been used on the same instrument for more than 60 days.

Refer to the Chapter of “Calibration” in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about calibration.

Control Testing

Sexual Hormone Controls are matched with E2 reagent pack. There are two levels of controls: Low Control (L) and High Control (H).

Test two levels of controls with E2 reagent on the instrument within 24 hours before testing real samples. Control testing is highly recommended every time the lot of reagent has been changed, the instrument has been re-calibrated, or after trouble shooting/ maintenance service.

Before the control testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put controls on a sample rack, and load the sample rack into the instrument; Select “Control” on the interface of test menu, select test name and control lot; Click on “Start” and begin the testing. Check the results after the control test is finished.

Control test results should fall into a specific range. If it is out of the target range, the user should check the system, such as expiration date of the controls, storage condition, instrument performance and status. After root cause analysis and corrective action, the user should test controls again. If the same problem exists, please contact customer service of Medcaptain.

Each laboratory should set up its own control range and frequency of control testing, based on its own practice. Refer to the Chapter of “Control Testing” in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about control testing.

Sample Testing

Before sample testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date, etc.).

If a sample collection tube is directly loaded to the instrument for testing, the sample volume should be at least 1.0 mL; Un-cap sample collection tubes, put samples on a sample rack, and push the sample rack into the instrument; Select “Sample” on the interface of test menu, enter information of samples, select test name; Click on “Start” and begin the testing. Check the results after the sample test is finished.

The reagent usage for each test is: R1 50 μL, R2 100 μL, and R3 150 μL. The instrument aspirates and mixes each component in the reagent cartridge and incubates at 37 ℃. Time duration from sampling to result is about 30 min.

Refer to the Chapter of “Sample Testing” in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about sample testing.

Result Calculation

Based on the built-in calibration curve, the instrument automatically calculate E2 concentration of each sample, either in a unit of ng/L or pmol/L. Unit conversion factor: 1.0 ng/L = 3.67 pmol/L.

[Reference Intervals]

Samples for the study of reference intervals come from local area in Guangdong Province. A total of 528 healthy and normal people have been recruited (Male: 126; Female: 402), age ranges from 14 to 88 years old. Studies with the E2 assay have revealed the following E2 values:

Test subjects	Number	E2 (ng/L)		
		Percentile		
		50^{th}	5^{th}	95^{th}
Men	126	24.3	11.8	43.5

Women	Follicular phase	122	52.3	31.5	93.2
	Ovulation phase	35	202.3	61.2	534.5
	Luteal phase	124	115.3	62.5	229.8
	Postmenopause	121	<5	<5	135.3

Due to the differences in geography, race, sex, and age of tested population, the reference interval may vary in different laboratories. It is highly recommended for each clinical lab to establish its own reference intervals.

[Interpretation of Test Results]

The test data is for clinical reference only. It cannot be used as the solely confirmatory evidence nor to eliminate the possibility of diseases. Clinical diagnosis of patients should take clinical symptoms, body sign, disease history, other lab test results, and treatment response into comprehensive consideration.

Due to the risk of cross reactivity, this assay should not be used when monitoring Estradiol levels in patients being treated with Fulvestrant.

Steroid drugs may interfere with this test.

The measurement range of this assay is: 5~3000 ng/L. If estradiol concentration is lower than LoD, it will be reported as <5 ng/L; If estradiol concentration is over the upper limit, it will be reported as >3000 ng/L.

When the instrument shows a warning sign of "SMPL", it means there is insufficient sample volume. Make sure enough sample is added for repeating the test. When the instrument shows a warning sign of "SMPJ", it means the sample probe has been blocked. Clean sample clot in the probe before repeating the test. Some results are tagged with other signs. Refer to the Chapter of "Result Signs" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about results tagged with signs.

[Limitation of the Test Method]

The test data is for clinical reference only. It cannot be used individually as the evidence to confirm or eliminate the possibility of diseases.

For each endogenous interference substance with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

Endogenous interference substance	Concentration of interference substance
Total Protein	≤12 g/dL
Bilirubin	≤20 mg/dL
Hemoglobin	≤500 mg/dL
Triglyceride	≤1000 mg/dL

For potential cross-reactant with the concentration value shown in the table below, cross-reaction rate is also listed in the same table.

Cross-reactant	Concentration (ng/L)	Cross-reactivity
Mesterolone	100000	0.21%
Estrone	100000	6.62%
Estriol	100000	0.87%
Dihydrotestosterone	100000	0.07%
2-Methoxyestradiol	100000	0.46%
Testosterone	100000	0.04%
Danazol	100000	<0.01%
Progesterone	100000	0.01%
Ethisterone	100000	0.17%

Heterophilic antibodies in human serum may react with immunoglobulin in the reagent, and interfere with immunoassay *in vitro*. More clinical or diagnostic information is needed to confirm disease diagnosis of patients. Some patients have frequent contact with animals, or have been treated or diagnosed with mouse monoclonal antibodies. They may have generated heterophilic antibodies. For example, some patients under monoclonal antibody treatment may have human anti-mouse antibodies (HAMA) in blood circulation, leading to false positive or false negative results. Anti-interference components are added to this reagent formulation to minimize the impact of HAMA and ANA, but the problem may not be totally eliminated, and some sample testing may still be impacted. More clinical and diagnostic information is needed to make a solid conclusion.

Samples with a titer of no less than 1:1000 by anti-nuclear IgG test kit (indirect immune-fluorescence method) were studied in interference tests. It has shown less than ±10% error in testing results. For RF at a concentration of less than 1500 IU/mL, and for multiple representative human HAMA samples, the measurement error cause by the interference is within ±10%.

[Property and Performance]

1 Limit of Blank

LoB ≤3 ng/L.

2 Limit of Detection

LoD ≤5 ng/L.

3 Accuracy

Accuracy should meet at least one of the following criteria:

a) Test the accuracy reference samples at two levels multiple times respectively. Relative deviation between the measurement result and the target value must not exceed 10.0%.

b) Spike estradiol of a known concentration into samples at different estradiol levels. Spiked recovery should be 100.0%±15.0%.

4 Linearity

Test estradiol samples with concentration in the range of 5~3000 ng/L, the linearity correlation coefficient r≥0.9900.

5 Repeatability

Coefficient of variation (CV) for the test results of low (35±7 ng/L) or high (200±40 ng/L) corporate reference sample is less than 8.0%.

6 Lot-to-lot Variation

Coefficient of variation (CV) for the test results of low (35±7 ng/L) or high (200±40 ng/L) corporate reference sample of estradiol is less than 10.0%.

7 Accuracy of Calibrator Value Assignment

Use primary calibrators with assigned values from higher level measurement procedure, calibrate the immunoassay analyzer, and use the same lot of reagent to measure the value of each product calibrator. The measured value of Calibrator C1 or C2 has a relative deviation within ±10.0% from its assigned value.

8 Homogeneity of Calibrators

8.1 Within-vial Homogeneity

Within-vial homogeneity of Calibrator C1 or C2 is represented with coefficient of variation, and CV≤8.0%.

8.2 Between-vial Homogeneity

Between-vial homogeneity of calibrator C1 or C2 is represented with coefficient of variation, and CV≤5.0%.

[Attention Notes]

1 It is for *in-vitro* diagnosis only.

2 It can only be used by professionals.

3 Never use expired reagent kit.

4 Never mix components from different kits, or from different reagent lots.

5 Do not put the reagent cartridges upside down.

6 Detection of estradiol in a sample using different detection systems may yield different results, due to the difference in test methods, assay specificity, and factors of interference. The measured values from different systems should not be directly compared to avoid inappropriate clinical interpretation.

7 Strictly follow the protocol in the package insert, and operate according to the lab guidelines.

8 The test results can only be used for clinical reference. Clinical diagnosis of patients should take symptoms, body sign, disease history, other laboratory test results, and response to treatment for comprehensive assessment.

9 User should wear gloves and lab coat. Rinse with water if the skin is in contact with the reagent. Flush eyes with copious of water if eyes are in touch with the reagent, and see a doctor immediately.

10 Take all samples and reaction waste as potential biohazard. All waste must be handled following the local government regulation.

11 This product is a single-use cartridge. Reagent cartridges should be put back into refrigerator and stored at 2~8℃ if they have been placed at room temperature but not opened yet.

[Interpretation of Signs]

	Temperature limit.		Date of manufacturing
	*In vitro* diagnostic medical device		Catalogue number
	Batch Code		Consult instructions for use or consult electronic instructions for use
	Use-by Date		Authorized representative in the European Community/European Union
	This way up		CE marking
	Manufacturer		Unique device identifier
	Biological risks		

[References]

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[Basic Information]

MEDCAPTAIN MEDICAL TECHNOLOGY CO., LTD.

12th Floor, Baiwang Research Building, No. 5158 Shahe West Road, Xili, Nanshan, 518055 Shenzhen, Guangdong, PEOPLE'S REPUBLIC OF CHINA

Telephone: +86-755-26953369

Website: http://www.medcaptain.com

CE

Post-sales service: MEDCAPTAIN MEDICAL TECHNOLOGY CO., LTD.

Telephone: +86-755-26953369

Postal code: 518055

Manufacture Location: Building C, Jiale Science and Technology Industrial Park, Matian Street, Guangming, 518106 Shenzhen, Guangdong, PEOPLE'S REPUBLIC OF CHINA.

R Sight B.V.

Roald Dahllaan 47, 5629 MC, Eindhoven. The Netherlands

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