Human Estradiol CLIA Kit

Chemiluminescence Immunoassay for the quantitative determination of estradiol in human serum

REF SKT-019 CE IVD 100, 150 +2 +8 °C

INTENDED USE

Human Estradiol CLIA Kit is a Chemiluminescence Immunoassay (CLIA) intended for the quantitativemeasurement of human estradiol concentration in serum.

For in-vitro diagnostics purposes only

SUMMARY OF PHYSIOLOGY

Estradiol(E2) is a natural estrogen with a molecular weight of 272.3 Dalton. In general estradiol is secreted mainly by sheath and granulosa cells and corpus luteum during follicular development in non-pregnant women. During gestation, estradiol is secreted mainly by the placenta. In addition, the adrenal glands and men's testes are also thought to produce small amounts of estradiol. After estradiol enters the bloodstream, 1-3% of estradiol is free and does not bind to protein, and 97-99% binds to protein. Forty percent of estradiol is bound to sex hormone-binding globulin (SHBG) and the rest to albumin.[1, 2]

Estradiol levels are lowest and relatively constant in the early follicular stage, and rise in the late follicular stage. A rise in estradiol levels causes a rise in luteinizing hormone, triggering ovulation and entering the luteal phase. Estradiol levels rise during the luteal phase and peak about 8 days after ovulation. If the egg is unfertilized, estradiol levels drop and the next menstrual cycle begins.[3]
Estradiol levels, which reflect follicular maturity, are valuable for monitoring ovulation, analyzing sexual development, the causes of menstruation, infertility and menopause. Normally levels of estradiol in men are low and can be used to detect male-female syndrome.[4]

ASSAY PRINCIPLE

The Human Estradiol CLIA Kit is designed, developed, and produced for the quantitative measurement of human E2 level in serum samples. The assay utilizes the competitive binding mechanism for testing.

The calibrators, controls, or sample, monoclonal antibody to E2 labeled with microparticles are mixed and incubated, forming an antigen-antibody immuno-complex. Acridinium ester labeled E2 analog unbound to the sample binds to the Magnetically microgranular E2 monoclonal antibody , After precipitation in a magnetic field, the supernatant is decanted, and then a wash cycle is performed to remove any remaining substances that are not bound to the magnetic microbeads. Subsequently, the washed compound is sent into the measurement chamber where trigger solution is automatically added to initiate a chemiluminescence reaction.

The light signal is measured by a photomultiplier as relative light units (RLUs), which is inversely proportional to the concentration of E2 present in the sample.
The test result is automatically calculated by the system according to the working curve.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2℃~8℃ upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.It can be stored for 1 month at 2℃~8℃ after kit opening.

1. E2 Magnetic Particle Solution (01901)
Qty: 6.0 mL (100/kit),8.5 mL (150/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

2. Acridinium ester E2 (01903)
Qty: 6.0 mL (100/kit),8.5 mL (150/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. E2 Calibrators (01906-01908)
Qty: 3 x vials
Storage: 2 – 8°C
Preparation: Ready to Use
After the first use, it is recommended to storage at 2 - 8℃ and can be used within one month. Do not freeze.

4. E2 Controls (01909-01910)
Qty: 2 x vials
Storage: 2 – 8°C
Preparation: Ready to Use
After the first use, it is recommended to storage at 2 - 8℃ and can be used within one month. Do not freeze.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in New Zealand. It was obtained only from healthy donor animals, maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ECL100 Immunoassay Analyzer (ECL100) or ECL25 Immunoassay Analyzer (ECL25)
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. EDI™ Wash Reagent (P-594)
4. EDI™ Trigger Solutions A and B (P-595A, P-595B)

The instrument must operate using materials supplied by Epitope Biotechnology, Co.,Ltd. or Epitope Diagnostics, Inc. When materials sourced from third-party suppliers are being used, Epitope Biotechnology, Co.,Ltd. and Epitope Diagnostics,Inc. takes no responsibility for the validity of obtained results. Materials are available to purchase from

Epitope Biotechnology, Co.,Ltd. and Epitope Diagnostics, Inc. Please contact your distributor for more information.

SPECIMEN COLLECTION AND PREPARATION

1. Blood sample should be collected under sterile conditions.
2. For human serum samples only; other body fluids and samples may not yield accurate results.
3. Clinical samples should be tested within 2 hours after collection. If the measurement cannot be completed within 2 hours, please store under the following conditions:
   - storage at low temperature and away from light (2℃~8℃) for 2 days,
   - storage at -20℃ or below for 6 months
   - Freeze and thaw only once
4. Avoid heat-inactivated samples. Mixed, contaminated and hemolysis samples should be discarded.
5. Samples should be restored to room temperature before testing. Frozen samples should be completely melted and mixed well before use. Due to possible volatilization, samples, calibrators and controls on the ECL platform should be tested within 2 hours.
6. Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:
   - bilirubin: 10 mg/dL
   - triglyceride: 1800 mg/dL
   - hemoglobin: 500 mg/dL
   - biotin: 220 nmol/L
   - For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after taking the last dose of biotin.
7. A single assay of this item requires 80 µL of sample. This quantity does not include the amount of dead volume in the sample container, the capacity required for retesting, and other measurement items. For the definition of minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. Calibration is required for the first time use of a reagent lot and every 14 days thereafter or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seals on each container on the cartridges and insert soft caps.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance. For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber. The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
E2 Controls (01909-01910)	80	-
Samples	-	80
E2 Magnetic Particle Solution (01901)	50	50
Incubate at 37°C for 20 minutes		
Acridinium ester E2 (01903)	50	50
Incubate at 37°C for 10 minutes		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	100-200	100-200
Trigger Solution B (P-595)	100-200	100-200

NOTE FOR ASSAY PROCEDURE

Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there is no air bubble in any reagents, calibrator and control vials.

All the reagents in this kit are ready-to-use. Different lots of the same reagents are not inter-changeable and must not be used.

Please read the reagent instructions and equipment instructions carefully before using this kit and perform the test according to relevant requirements. When reagents are loaded, the equipment will automatically stir the magnetic particles to resuspend them. Allow the regent to mix for minimum 15 min before starting the assay program.

INTERPRETION OF RESULTS

1. The default result unit for estradiol project is PG /mL or pmol/L

(Conversion factor: pg/mL×3.671=pmol/L)

2. Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers, so direct comparisons should not be made to avoid false interpretation.

3. When the concentration of E2 in the sample exceeds 4800 pg/mL, the sample can be diluted (2 times is recommended) before detection.

4. When the sample concentration of E2 is lower than the detection lower limit, the test result will be reported as <20.00

pg/mL. When the sample concentration is higher than the detection upper limit, it will be reported as >4800 pg/mL.

EXPECTED VALUES

Patient Group		Reference range （pg/mL）
Male		20.00-47.00
Postmenopausal women		20.00-40.00
Non-pregnant woman	Follicles mid -	27.00-122.0
	Corpus luteum mid -	49.00-291.0
	ovulatory cycle	95.00-433.0

Note: each Laboratory is recommended to determine and establish its own reference range with local population.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 Immunoassay Analyzer or ECL 25 Immunoassay Analyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different kit lot numbers should not be combined or interchanged.
3. Test results obtained from the proposed kit should not be served as a sole basis for clinical diagnosis or patient management.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measured value is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

1. **Limit of Detection(LoD):**
   - ≤ 20.00 pg/mL
2. **Linearity:**
   - 20.00 pg/mL ~ 4800 pg/mL
   - linearity correlation coefficient R ⩾0.990
3. **Accuracy:**
   - relative deviation within ±15%
4. **Precision:**
   - Intra-assay repeatability: CV≤8%
   - Inter-assay reproducibility: CV≤15%

NOTES

1. Read the instructions carefully and gently but thoroughly mix the reagent before use. Remove any air bubbles before loading the reagents onto the equipment.
2. Keep the reagent in the storage conditions indicated in this IFU and on the reagent label. Do not freeze reagents.
3. Avoid contact with skin, eyes and mucous membrane. Upon contact, flush the area with clean water immediately.
4. All patient samples must be treated as potential infectious material.
5. Components in different kits cannot be mixed.
6. All waste must be disposed of in compliance with local regulations and laws.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Biotechnology Co,Ltd and its distributors DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Biotechnology Co,Ltd.. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCE

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3. Lorick KL, Jensen JP, Fang S, et al. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. ProcNatlAcadSci U S A., 1999, 96(20): 11364-11369.
4.Jensen T, Bildt MVD, Dietz HH, et al. Another phocine distemper outbreak in Europe. Science, 2002, 297(5579): 209.

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or to place an order, please contact Epitope Diagnostics, Inc. in USA at +1 858-693-7877 or email to cs@epitopediagnostics.com

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

In Vitro Diagnostic Device

European Conformity

Lot Number

Catalog Number

Read Instructions before Use

Number of Tests

Store at

Use by

Keep Away from Heat and Direct Sun light

Manufacturer

Authorized Representative in Europe

Distributor