

TISENC

Estradiol (CLIA) PACKAGE INSERT



INTENDED USE

Estradiol (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of circulating Estradiol (E2) in human serum or plasma, which is intended as an aid to diagnosis of ovarian disease.

The assay can be used for samples over the range of 15pg/mL-3000pg/mL.

The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120 and other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Estrogens are responsible for the development of the secondary female sex characteristics. Together with gestagens they control all the important female reproductive processes.

The biologically most active estrogen is 17β-estradiol. This is a steroid hormone having a molecular weight of 272 Daltons. Estrogens are produced primarily in the ovary (follicle, corpus luteum), but small quantities are also formed in the testes and in the adrenal cortex.

During pregnancy, estrogens are mainly formed in the placenta. In human plasma the bulk of estradiol is bound specifically to SHBG (= sex hormone binding globulin)¹ and non-specifically to human serum albumin. Estrogen secretion is biphasic during the menstrual cycle.² The determination of estradiol is utilized clinically in the elucidation of fertility disorders in the hypothalamus-pituitary-gonad axis, gynecomastia, estrogen-producing ovarian and testicular tumors and in hyperplasia of the adrenal cortex. Further clinical indications are the monitoring of fertility therapy and determining the time of ovulation within the framework of invitro fertilization (IVF)^{3,4,5}.

ASSAY PRINCIPLES

The principle of Estradiol (CLIA) hereinafter also referred to as the “E2” combines a competitive immunoassay method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as following:

Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridges. All of the assay steps are performed automatically by the ACCRE analyzer.

The sample is manually pipetted into the sample well of reagent cartridge. The sample, the sample treatment solution and the anti-E2 antibody coated paramagnetic microparticles are mixed and incubated, and the antigen in the sample is released by the release agent and combined with the anti-E2 antibody to form an antibody-antigen complex.

The estradiol-alkaline phosphatase conjugate is added to the mixture, and incubated, and the estradiol-alkaline phosphatase conjugate competes with the antigen in the sample for binding sites on a limited amount of specific anti-E2 antibody.

After the reaction was completed, the magnetic field adsorbed the magnetic beads and the unbound material was removed by three washings. After the reaction substrate was added, the luminescence intensity was measured by a photomultiplier tube. The concentration of the substance to be detected in the sample is inversely proportional to the intensity of the luminescence. At the end, the results were automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

CONTENT OF THE KIT

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls

The calibrator in the kit has been traced to Roche fully automated chemiluminescence immunoassay analyzer cobas e411 and Elecsy Estradiol.

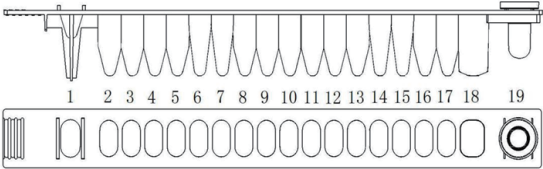
For specific calibrator concentration values, see the Calibrator Barcode Sheet for each lot.

Contents	Quantities				Notice
	[REF] W40A	[REF] W40C	[REF] W48A	[REF] W48C	
E2 Reagents Cartridges	60 strips	36 strips	60 strips	36 strips	Ready to Use
E2 Calibrator 1 (CAL1)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Phosphate buffer, 50mM; ProClin300, 0.48g/L; BND, 0.2g/L
E2 Calibrator 2 (CAL2)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Phosphate buffer, 50mM; ProClin300, 0.48g/L; BND, 0.2g/L; Estradiol antigen, 100pg/mL
E2 Calibrator3 (CAL3)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	Phosphate buffer, 50mM; ProClin300, 0.48g/L; BND, 0.2g/L; Estradiol antigen, 1500pg/mL
E2 Quality Control Level 1 (QC1)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Phosphate buffer, 50mM; ProClin300, 0.48g/L; BND, 0.2g/L; Estradiol antigen, 100pg/mL Only for the configuration with Quality Controls
E2 Quality Control Level 2(QC2)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	Phosphate buffer, 50mM; ProClin300, 0.48g/L; BND, 0.2g/L; Estradiol antigen, 500pg/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configurations with Quality Controls

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

Reagent Cartridge

The strip consists of 19 wells. The 1st well is the sample well and the last well 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a label, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridge. And please do not interchange integral component from different reagents or lots.



Description of The Reagent Cartridge

Well	Component	Content	Volume
5	Sample Treatment Solution (R3)	Tris buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
7	Magnetic Particles(R1)	Anti-E2 antibody coated magnetic particles, 0.2g/L; Tris Buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
9	Enzyme Label (R2)	Estradiol-alkaline phosphatase conjugate, 3.0mg/L; MES Buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	50μL
10, 11, 12	Wash Solutions	Tris Buffer, 50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	350μL
16	Substrate	APS-5	100μL

Note: Well 1, 2, 3, 4, 6, 8, 13, 14, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc)
- Pipette with disposable tip to dispense 200μL.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Estradiol (CLIA) kits including reagent cartridges, calibrators and quality controls **upright** at 2~8°C in dark place and the kit can be valid for 18 months from manufactured date.
- After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- When opened, the calibrators will be stable for 30 days at 2~8°C, and 3 months at -20°C and below -20°C. Calibrators can only be frozen and thawed once. Quality controls will be stable for 30 days at 2~8°C.
- Keep away from sunlight.
- Do not freeze reagent cartridges.

SAMPLES

The sample volume for Estradiol (CLIA) assay is 80μL, please note that at least 100μL of sample has to be pipetted manually into the reagent cartridge.

Sample type and collection

Human serum or plasma (Heparin sodium, EDTA-K₂ or EDTA-K₃) are recommended.

Types of tubes:

- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin sodium,
- Plastic tube with EDTA-K₂.
- Plastic tube with EDTA-K₃.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.

It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation

The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.

For use of the sample tubes, follow the tube manufacturer's instructions for use.

The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.

Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.

Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.

If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.

Severe hemolysis samples (hemoglobin >500mg/dl) or heat-inactivated samples are not recommended for applying on the reagents.

Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.

Mix using with a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability

Samples (serum and plasma) can be stored at 15~30°C for up to 8 hours or at 2~8°C for up to 48 hours; if longer storage is required, freeze the serum or plasma at -20°C or below -20°C for up to 6 months, without exceeding 1 freeze/thaw cycle.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.

When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.

When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer.

Calibration is necessary under the following conditions:

- (1) New reagent lot number applied;
- (2) **Same lot of reagents used more than 4 weeks;**
- (3) Quality Control out of control;
- (4) When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure

1. Remove the kit from storage at 2-8°C and take out the required number of reagent cartridge. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay E2 strips for each sample, calibrator or control respectively.
3. Place the reagent cartridge into the reagent rack.
4. Manually pipette the Sample, Calibrator, or Controls into the sample well of each reagent cartridge.

Please note that at least 100µL of sample has to be pipetted manually into the reagent cartridge and 80µL sample is needed for each test.

*** Before pipetting: Mix the samples using a vortex-type mixer if necessary. and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.**

5. Put the sample rack into the reagent chamber of the ACCRE analyzer.
6. Place the assay tips into the reagent chamber of the ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by the USER MANUAL.
8. Estradiol (CLIA) assay will be completed within approximately 23 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer . Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 3000pg/mL can be diluted with negative serum or plasma manually. The recommended dilution is 1:2. And the concentration after dilution should be more than 500pg/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality Control

Quality control can be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 3-point calibration, and calculated according to a predefined mathematical model, the results expressed in pg/mL. The results can be converted as the unit of ng/L or pmol/L by manually through below conversion factors.

Conversion factors: pg/mL×1=ng/L, pg/mL×3.67=pmol/L, pmol/L×0.273=pg/mL.

Interpretation of Results

The detection range is from 15 pg/mL to 3000pg/mL. For samples with E2 below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <15pg/mL; if the sample is higher than the upper limit of detection, the reported result is >3000pg/mL.

Results of Estradiol (CLIA) assays are determined by testing serum specimens from a study in clinical centers with group of totally 688 healthy adult individuals in China Guangdong (138 males and 550 females). The 95th percentile value is as follows:

Group		<Cases	2.5 th - 97.5 th Percentile value (pg/mL)
Healthy Males		138	<48.83
Females (Non pregnancy)	Follicular Phase	138	<206.6
	Period of Ovulation	135	38.97~ 410.5
	Luteal Phase	142	22.92~ 326.3
	Post menopause	135	<107.8

*** Please note that results may differ between laboratories due to variations in**

population and test method. If necessary. each laboratory should establish its own reference range.

In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

LIMITATIONS

1. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
2. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
3. Please note that heterophile antibody in human serum or plasma can interfere the test result.
4. High concentration of HAMA can also cause a false positive or false negative result.

PERFORMANCE CHARACTERISTICS

Detection limits

LOD (Limit of Detection) =15pg/mL.
LOD was determined according to CLSI ® EP17-A2 recommendations.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).
The measurement range of the Estradiol (CLIA) assay is: 15 pg/mL ~ 3000pg/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The Estradiol (CLIA) assay is linear between 15pg/mL to 3000pg/mL. The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision

A precision study was performed according to CLSI ® EP05-A3 recommendations. The Within-lot precision CV% is less than 10%.
The between-lot precision CV% is less than 15%.

Accuracy

When a known concentration of Estradiol (CLIA) assay high value sample is added to a low value serum sample, the recovery rate is between 85% and 115%.
The sample which is a control with trueness traceability is used for the detection, and the relative deviation between the detection result and the calibration concentration is within ±10%.

Interference

Potential interference substances were studied according to CLSI ® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤ 500mg/dL
Bilirubin	≤ 20mg/dL
Triglyceride	≤ 1500mg/dL
Total Protein	≤ 10g/dL
Rheumatoid factor	≤ 1000IU/mL

HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

WASTE DISPOSAL

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
	In vitro diagnostic medical device		Use-by date		Do not re-use
	Temperature limit		Batch code		Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use		Contains biological material of animal origin
	Biological risks		Caution		CE marking
	Upright		Fragile, handle with care		Do not roll
	Control				

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