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Instructions for use

Estrone ELISA

REF FR E-2300

2°C–8°C Σ 96 IVD CE

Estrone ELISA

1. INTRODUCTION

1.1 Intended use

The **Estrone ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Estrone in serum and EDTA plasma.

1.2 Summary and Explanation

There are three forms of estrogens in the female body: estrone (E1), estradiol (E2), and estriol (E3. The estrogens are involved in the development of female sex organs and secondary sex characteristics and effect most organ systems, including brain, breast, cardiovascular (heart and vasculature), immune, reproductive (ovaries and uterus), bladder, skin, and bone (1,11).

Estrone is produced primarily from androstenedione. In premenopausal women, more than 50% of the estrone is secreted by the ovary. In pre-pubertal children, men and postmenopausal women, the major portion of estrone is derived from peripheral tissue conversion (2). During the follicular phase of the menstrual cycle, the estrone level increases with a peak around day 14 and decreases thereafter. A second peak during the luteal phase occurs around day 21 of the cycle. These changes of estrone concentration mirror the estradiol levels (3). Up to week 4-6 of pregnancy, estrone originates primarily from maternal sources but gradually increases during week 6-10 of pregnancy due to placental secretion of estrone (4). After menopause, estrone levels do not decline as dramatically as estradiol levels. In postmenopausal women, estrone is the major estrogen. In males the concentration of E1 has been reported to increase with age inversely to that of 17-OH-progesterone (5,9).

In premenopausal women estrone levels can increase after conversion of large amounts of androstenedione produced in polycystic ovary syndrome (6) and ovarian tumors. Furthermore, excess weight results in increased estrogen concentrations from peripheral conversion of androgens to estrogens (mainly E1) in adipose tissue by aromatase enzyme (10).

This test can be used for monitoring low-dose female hormone replacement therapy in post-menopausal women, monitoring of anti-estrogen therapy (e.g. with aromatase inhibitors), and as an adjunct to clinical assessment, imaging studies and bone mineral density measurement in the fracture risk assessment of postmenopausal women. In addition, it can be useful as part of the diagnosis and work-up of precocious and delayed puberty in females (to a lesser degree in males), and of suspected disorders of sex steroid metabolism (e.g. aromatase deficiency and 17 alpha-hydroxylase deficiency).

2. PRINCIPLE OF THE TEST

The Estrone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with a polyclonal rabbit antibody directed towards an antigenic site of the Estrone molecule. Endogenous Estrone of a patient sample competes with an estrone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estrone in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Estrone in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C - 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

9. Allow the reagents to reach room temperature (21 °C - 24 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4. REAGENTS

4.1 Reagents provided

FR E-2331 96 **Microtiterwells**

Contents: 12x8 (break apart) strips, 96 wells, Wells coated with anti-Estrone antibody (polyclonal)

Standards and **Controls** - Ready to use

Cat. no.	Component	Concentration pg/ml	Volume / Vial
FR E-2301	STANDARD A	0	1 ml
FR E-2302	STANDARD B	15	1 ml
FR E-2303	STANDARD C	30	1 ml
FR E-2304	STANDARD D	90	1 ml
FR E-2305	STANDARD E	270	1 ml
FR E-2306	STANDARD F	810	1 ml
FR E-2307	STANDARD G	2400	1 ml
FR E-2351	CONTROL 1	For control values and ranges please refer to vial label or QC-Report.	1 ml
FR E-2352	CONTROL 2		1 ml

Conversion: pg/ml x 3.69 = pmol/l

Contents: contain non mercury preservative.

The standards are calibrated against certified reference material code E-075 (Cerilliant).

FR E-2340 CONJUGATE **Enzyme Conjugate** - Ready to use

Contents: Estrone conjugated to horseradish peroxidase
contains non-mercury preservative

Volume: 1 x 14 ml/vial

FR E-0055 SUBSTRATE **Substrate Solution** - Ready to use

Contents: Tetramethylbenzidine (TMB)

Volume: 1 x 14 ml

FR E-0080 STOP-SOLN **Stop Solution** - Ready to use

Contents: contains 0.5 M H_2SO_4

Volume: 1 x 14 ml/vial

Hazards identification: 

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

FR E-0030 WASH- CONC 40x **Wash Solution -** Concentrated 40x

Volume: 1 x 30 ml/vial
See "Reagent Preparation"

Note: Additional *Standard A* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (21 °C - 24 °C) prior to use.

Wash Solution
Add deionized water to the 40X concentrated *Wash Solution*.
Dilute 30 ml of concentrated *Wash Solution* with 1170 ml deionized water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SPECIMEN COLLECTION AND PREPARATION

Serum or EDTA plasma can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 4 days at 2 °C - 8 °C prior to assaying.
Specimens held for a longer time (up to 18 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard A and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:
a) dilution 1:10: 10 µl sample + 90 µl Standard A (mix thoroughly)
b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Standard A (mix thoroughly).

6. ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- **The test should not be performed above a temperature of 24 °C.**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **25 µl** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3.	Dispense **100 µl *Enzyme Conjugate*** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4.	Incubate for **60 minutes** at room temperature.
5.	Briskly shake out the contents of the wells. Rinse the wells **5 times** with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets. ***Important note*:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6.	Add **100 µl** of ***Substrate Solution*** to each well.
7.	Incubate for **15 minutes** at room temperature.
8.	Stop the enzymatic reaction by adding **50 µl** of ***Stop Solution*** to each well.
9.	Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4 - Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 2400 pg/ml. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard A (0 pg/ml)	2.24
Standard B (15 pg/ml)	2.04
Standard C (30 pg/ml)	1.88
Standard D (90 pg/ml)	1.48
Standard E (270 pg/ml)	1.05
Standard F (810 pg/ml)	0.68
Standard G (2400 pg/ml)	0.43

7. EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the Estrone ELISA the following values are observed:

Population	n	range (min. – max.) (pg/ml)	Mean (pg/ml)	2.5th - 97.5th Percentile (pg/ml)	Median (pg/ml)
Males	60	9.0 - 79.1	47.6	15.6 - 77.0	47.9
Females					
premenopausal	110	11.2 - 338.3	69.4	15.5 - 220.2	52.8
postmenopausal	91	9.4 - 59.6	28.6	11.0 - 54.5	27.4

These results correlate well to published data (8, 9).

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8. QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC Report added to the kit. The values and ranges stated on the QC Report always refer to the current kit lot and should be used for direct comparison of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

9. PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 8.1 - 2400 pg/ml.

9.2 Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Compound	Cross Reactivity (%)
17-OH-Progesterone	0.01
Androstenedione	0.04
Corticosterone	0.01
Cortisone	0.39
DHEA	0.01
Estradiol	1.19
Estriol	0.07
Estrone 3-β-D-glucoronide	0.35
Estrone 3-sulfate	0.44
Ethisterone	0.39
Hydrocortisone	0.30
Progesterone	< 0.01
Testosterone	0.03

9.3 Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Zero Standard (Standard A) and was found to be 8.1 pg/ml.

The limit of detection (LoD) of the assay is 14.6 pg/ml.
The limit of quantification (LoQ) of the assay is 15.8 pg/ml.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	10	57.68	8.4
2	10	61.00	6.7
3	10	82.14	6.8
4	10	409.13	6.4

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (pg/ml)	CV (%)
1	30	32.98	11.9
2	30	61.91	9.7
3	30	81.96	7.4
4	30	451.6	8.7

9.4.3 Inter-Lot

The inter-assay (between-lots) variation was determined by repeated measurements of 3 samples in 3 different kit lots.

Sample	n	Mean (pg/ml)	CV (%)
1	18	2005.49	4.8
2	18	1122.06	2.3
3	18	66.28	14.0
4	18	53.46	8.4

9.5 Recovery

Samples have been spiked by adding Estrone solutions with known concentrations in a 1:1 ratio.
The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous Estrone + added Estrone) / 2; because of a 1:2 dilution of serum with spike material).

Sample		1	2	3	4	5	6
Sample Type		Serum	EDTA	EDTA	Serum	Serum	Serum
Concentration [pg/ml]		31.95	32.06	33.83	46.08.	464.78	639.70
Average Recovery [%]		102.0	103.4	93.3	108.0	105.6	92.4
Range of Recovery [%]	**from**	96.0	96.1	85.7	101.7	95.9	89.2
	to	104.2	109.4	110.2	114.3	114.2	95.4

9.6 Linearity

Sample		1	2	3	4	5
Sample Type		**EDTA**	**EDTA**	**Serum**	**Serum**	**Serum**
Concentration [pg/ml]		92.42	210.70	496.00	547.96	1649.26
Average Recovery		110.3	101.4	106.0	96.6	101.6
Range of Recovery (%)	**from**	108.4	95.7	88.9	85.0	95.7
	to	112.1	105.9	113.4	109.0	105.2

9.7 Comparison Studies

For method comparison, 40 samples spanning the complete assay range were tested by the Estrone ELISA and by the RIA.
The resulting regression equation was:

ELISA = 0.743 RIA + 15.52; R^2 = 0.990

10. LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 7.5 mg/ml) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of estrone in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test.

11. LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacturer.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12. REFERENCES / LITERATUR

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number		