



ESTRONE ELISA



BL-09-E

IN VITRO DIAGNOSTIC USE

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1 INTRODUCTION

The Bio-Line **Estrone** Enzyme Immunoassay Kit provides materials for the quantitative determination of estrone in serum and plasma. This assay is intended for in vitro diagnostic use only.

Estrone is a steroid, like estriol and estradiol belonging to the class of Estrogens. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Before the ovum is fertilized the main action of the estrogens are the growth and function of the reproductive tract in order to prepare it for the fertilized ovum.

Estrone is produced primarily from androstenedione originating from either the adrenal cortex or gonads. In premenopausal women, more than 50 % of the estrone is secreted by the ovary. In prepubertal children, men and postmenopausal women, the major portion of estrone is derived from peripheral tissue conversion of androstenedione.

During the follicular phase of the menstrual cycle the estrone level shows a slight increase, the production of estrone then increases markedly to peak at around day 13. The peak is of short duration and by day 16 of the cycle levels will be low. A second peak occurs at around day 21 of the cycle. If fertilization does not occur then production of estrone decreases. These changes of estrone concentration are in parallel to that of estradiol.

After menopause, estrone levels do not decline as dramatically as estradiol levels.

In males the concentration of E1 rises up with age inversely to that of 17-OH-progesterone.

2 PRINCIPLE OF THE TEST

This assay is based on the competition principle and the microtiter plate separation. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After an incubation the wells are washed to stop the competition reaction. Having added the TMB substrate solution the concentration of antigen is inversely proportional to the optical density measured. The measured ODs of the calibrators are used to construct a calibration curve against which the unknown samples are calculated.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- 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.
- Avoid contact with Stop Solution containing 1 M H₂SO₄. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request.

4 KIT COMPONENTS

4.1 Contents of the Kit

The components contain ≤ 250 µl solution, please care that all the solution is on the bottom of the vial.

- 12x8 (break apart) strips, 96 wells
Wells coated with anti-Estrone antiserum (rabbit-polyclonal)
- | | |
|-----|---|
| CAL | N |
|-----|---|

 N=1 to 5
 5 vials (ready to use), 0.5 ml
 Protein based buffer with a non-mercury preservative
 See exact values on the vial labels
 To convert to pmol/l: pg/ml x 3.69 = pmol/l
- | | |
|-----|---|
| CAL | 0 |
|-----|---|

 1 vial (ready to use), 1.0 ml
 0 pg/ml, protein-based buffer with a non-mercury preservative
- | | | |
|------|------|------|
| BIOT | CONJ | CONC |
|------|------|------|

 Biotinylated – E1
 1 vial, 0.2 ml, concentrated
 in a stabilizer buffer with a non-mercury preservative
 Dilution see: Preparation of reagents
- | | | |
|------|-----|------|
| AVID | HRP | CONC |
|------|-----|------|

 1 vial, 0.2 ml, concentrated
 in a stabilizer buffer with a non-mercury preservative
 Dilution see: Preparation of reagents
- | | |
|-------|-----|
| CHROM | TMB |
|-------|-----|

 1 vial, 16ml, ready to use
 contains tetramethylbenzidine in buffer with stabilizers
- | | |
|-----|-----|
| ASS | BUF |
|-----|-----|

 1 vial; 15 ml; ready for use
 in a stabilizer buffer with a non-mercury preservative
- | | |
|---------|---|
| CONTROL | N |
|---------|---|

 1 vial, 0.7 ml, ready to use
 protein-based buffer with a non-mercury preservative
 For concentration range, see vial label
- | | |
|------|------|
| STOP | SOLN |
|------|------|

 1 vial, 6 ml, ready to use
 contains 1M H₂SO₄
 Avoid contact with the stop solution. It may cause skin irritations and

10	WASH	SOLN	CONC
	burns.		
	1 vial, 50 ml, concentrated		
	Buffer with detergent		
Dilute 1 : 10 with distilled water			

Note: Additional Zero Calibrator for Sample dilution available on request.

4.2 Equipment and material required but not provided

1. A microtiterplate calibrated reader (450±10 nm).
2. Calibrated variable precision micropipettes (Varipette Eppendorf), Multipette Eppendorf or similar products.
3. Absorbent paper.
4. Distilled water.

4.3 Storage and stability of the Kit

- When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Enzyme-Conjugate, Substrate Solution, Calibrators and Zero Calibrator must be stored at 2° to 8°C.
- Microtiter wells must be stored at 2° to 8°C. Once the foilbag has been open care should be taken to close it tightly again.

4.4 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Buffer

Dilute, for example, 20 ml of the concentrate 1:10 with distilled water up to 200 ml. This gives the ready for use Wash Buffer. Occasionally crystals will form. Warm solution to ensure all are completely dissolved before use.

Store the diluted Wash Buffer at 2 – 8 °C for up to two weeks.

Biotin and Avidin HRP Conjugate

Dilute both of the Biotin- and the Avidin-HRP conjugate concentrates 1:100 into the same assay buffer before use (e.g. 20 µl of each Biotin and Avidin-HRP in the same 2 ml of Assay Buffer) If the whole plate is to be used dilute 0.12 ml of each conjugate concentrate in 12 ml of assay buffer. Prepare the conjugate **at least 20 to 25 minutes prior to pipetting** only for the actual test run and discard any that is left over.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets.

4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, Bio-Line Europe have to be informed written, 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

5.1 Specimen collection

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not use haemolytic, icteric or lipaemic serum.

5.2 Specimen storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimen held for a longer time 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 serum specimen is found to contain more E1 than the highest calibrator, the specimens can be diluted 10-fold with Zero Calibrator and reassayed as described in Assay Procedure.

6 TEST 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.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipet tips for each calibrator, control of sample in order to avoid crosscontamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

6.2 Procedural Notes

- All calibrators, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
- The concentration of the samples can be read directly from this calibrator curve. Samples with a concentration higher than that of the highest calibrator have to be diluted 1 : 10 with Zero Calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3 Assay Procedure

1. Secure the desired number of Microtiterwells in the holder.
2. Dispense **50 µl** of each calibrator, control and samples **with new disposable tips** into appropriate wells.
3. Dispense **100 µl** Enzyme Conjugate into each well.
4. Incubate for **1 hour** on a plate shaker (500 rpm) at room temperature (18-24°C).
5. Briskly shake out the contents of the wells.
6. Wash the wells 4 times with **300 ul Wash Buffer** (the use of a washer is recommended). Strike the wells sharply on absorbent paper to remove residual water droplets.

7. **Important note:**
8. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
9. Add **150 µl** of Substrate Solution to each well.
10. Incubate for **10-15 minutes** at room temperature on an orbital shaker until calibrator A attains a dark blue colour for desired OD.
11. Stop the enzymatic reaction by adding **50 µl** of TMB Stop Solution to each well.
12. Read the OD at **450±10 nm** with a microtiterplate reader **within 10 minutes** after adding the Stop Solution.

6.4 Calculation of Results

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Construct a calibrator curve by plotting the mean absorbance obtained from each calibrator against its concentration in IU/ml 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 of Estrone in pg/ml from the calibrator curve. Depending on experience and/or the availability of computer capability, other methods of data deduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this calibrator curve. Samples with Estrone concentration higher than that of the highest calibrator have to be diluted with zero calibrator. For the calculation of the concentrations this dilution factor has to be taken into account.

Below is listed a typical example of a calibrator curve with the Estrone ELISA.

calibrators (pg/ml)	OD (450 nm)
0	2.641
15	2.075
50	1.540
200	0.973
800	0.391
2000	0.236

7 ASSAY CHARACTERISTICS

7.1 Expected values

It is recommended that each laboratory establishes its own range of normal values. All participants in the normal range study were apparently healthy subjects. The normal value range is assumed to be as 95%-percentile.

	pg/ml
Females	25 – 350
Pregnancy	100 – 8000
Males	25 – 150

7.2 Specificity

The specificity of the estrone assay was assessed according to Abraham's method. The percentage indicate cross reactivity at 50 % displacement compared to estrone.

Component	% Cross-reactivity	Component	% Cross-reactivity
Estrone	100.0	16-Ketoestriol	0.5
17β - Estradiol	1.2	Androstenedione	< 0.09
Estrone-Sulfat	0.8	Testosterone	< 0.03
17α - Estradiol	0.4	19-Nortestosterone	< 0.02
Estriol	0.03	Ethinylestradiol	< 0.02

The following steroids were tested but show cross-reactivity < 0.01 %:

Cortisol, Cortisone, Corticosterone, 11-Deoxycortisol, DHEA, Progesterone, Pregnanetrone, 17-OH-Progesterone.

7.3 Sensitivity

The lowest detectable level that can be distinguished from the zero calibrator is 4 pg/ml (defined as mean of the ODs of the zero calibrator minus 2 SD) as read from the calibrator curve.

7.4 Accuracy

Quality Control

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 certificate added to the kit. The values stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy 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.

7.5 Precision

7.5.1 Intra Assay Variation

Within run variation was determined by replicate determination of two different control sera in one assay. The within assay variability is shown below:

Sample	n	Mean (pg/ml)	Standard Deviation(pg/ml)	CV (%)
1	20	55.4	4.5	9.1

2	20	250.5	16.8	6.7
3	20	1563.6	112.8	7.2

7.5.2 Inter Assay Variation

Between run variation was determined by replicate measurements of two different control sera in several different assay. The between assay variability is shown below:

Sample	n	Mean (pg/ml)	Standard Deviation (pg/ml)	CV (%)
1	10	55.6	6.5	11.7
2	10	260.1	18.8	6.9
3	10	1478.6	145.6	9.9

7.6 Recovery

Normal human sera with known concentrations were enriched with increasing amounts of estrone (all results given in pg/ml).

Sample	Added amount (pg)	Expected values (pg/ml)	Measured values (pg/ml)	Recovery %
1 (52 pg/ml)	200	252	315	125
	400	452	557	120
	1000	1052	1235	117
2 (75 pg/ml)	375	450	493	110
	750	825	890	108
	1500	1575	1754	111
3 (145 pg/ml)	375	520	502	97
	750	895	955	116
	1500	1645	1378	84

7.7 Linearity

Three samples having different concentration levels were serially diluted with zero calibrator and the estrone contents were assayed in the diluted samples by the ELISA. Three dilutions were performed for each sample.

Sample	Dilution	Measured conc. (pg/ml)	Expected conc. (pg/ml)	Recovery (%)
1	--	348		
	1:2	185	174	106
	1:4	100	87	115
	1:8	49	43,6	114
2	--	595		
	1:2	319	298	107
	1:4	153	149	103
	1:8	80	74	108
3	--	603		
	1:2	288	302	95
	1:4	144	151	95
	1:8	73	75	98

8 LIMITATIONS OF USE

8.1 Interfering Substances

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.

Azide and thimerosal at concentrations higher than 0.1 % interfere in this assay. Therefore control sera or samples containing higher concentrations of the above mentioned components may give false results.

Reagents from different kits or lots should not be mixed, due to possible different shipping or storage conditions.

Any improper handling of samples or modification of this test might influence the results.

Interferences caused by improper sample handling are explained in chapter 'Specimen Collection and Storage'.

For diagnostic purpose results obtained by this assay should be used in conjunction with other test results, the overall clinic presentation to the physician, and all other appropriate information.

9 LEGAL ASPECTS

9.1 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 calibrators 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.

9.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. 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 therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

9.3 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 9.2. 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.

10 REFERENCES

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