



Read entire protocol before use. Estradiol-Elisa

Estradiol, 17 β -Elisa BL-21- E

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

Not be used for calculation risk of Trisomie 21.

I. INTENDED USE

An Immunoenzymetric assay for the in vitro quantitative measurement of human ESTRADIOL in serum and plasma.

II. GENERAL INFORMATION

- A. Name: Bio-Line Estradiol -Elisa Kit
- B. Catalogue number: BL-21-E: 96 tests
- C. Manufactured by: Bio-Line S.A.
Rue André Fauchille.17 - B-1150 Bruxelles - Belgium
- D. For technical assistance or ordering information contact:
Tel: +32-2-736.62.18. Fax: +32-2-742.13.15.

III. CLINICAL BACKGROUND

17 β -estradiol (E2) is a C-18 steroid hormone (molecular weight 272.4) produced mainly by the ovary and placenta, and in small amounts by adrenals and testes. Estradiol is in equilibrium with estrone, which can be converted to estriol by the liver and placenta.

Like for LH, FSH, progesterone, measurement of estradiol concentration in serum, peritoneal fluid and follicular fluid is an essential biochemical tool for the investigation of fertility, tumor and sexual diseases, and disorders of hypothalamic/pituitary/gonadal axis.

For example:

- To detect the follicular phase;
- To check the effectiveness of the induction of ovulation (with ultrasound) and the level of E2 in follicular fluid. It allows normal detection or dysfunctional ovulation induction (the empty follicle syndrome may reflect a dysfunctional ovulation induction);
- To diagnose the luteinized unruptured follicle (LUF) syndrome (by the estimation of 17 β -estradiol and progesterone levels in the peritoneal fluid);
- To aid in the diagnosis of breast tumors (total estrogens -E1-E2- and 17 β -hydroxysteroid dehydrogenase activity are significantly higher in malignant than in non malignant breast tissues);
- With LH-FSH and E2-levels, it is possible to suspect a Stein Cohen-Leventhal syndrome;
- Other areas of investigation are: premature adrenarache, gynecomastie and menopausal period

IV. PRINCIPLES OF THE METHOD

The Bio-Line Estradiol-Elisa kit is an enzyme Immunoassay performed in microtiter plate.

A fixed amount of estradiol (E2) labelled with horseradish peroxydase (HRP) competes with unlabelled estradiol present in standards or samples for a limited number of binding sites of specific antibody (Ab.).

The (E2-HRP-Ab.) complex is simultaneously fixed on the wells of the microtiterplate coated with anti-rabbit-gammaglobulins in excess.

Nether extraction nor chromatography are required due to the high specificity of the Ab.

After 2 hours incubation at RT the microplate is washed to stop the competition reaction.

The revelation solution (tetramethylbenzidine (TMB)-H₂O₂) is added and incubated for 30 min. The reaction is stopped with H₂SO₄ and the microplate is read at appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is inversely proportional to the

estradiol concentration. A standard curve is plotted and estradiol concentration in samples are determined by interpolation from the standard curve.

V. REAGENTS PROVIDED

Contents of the Kit

- | | | |
|--------------|----------|------|
| TLJ | | |
| Ab | Anti -E2 | |
| CAL/Contr | 1 to 5 | |
| CAL | 0 | |
| Conj | HRP | |
| Conj buffer | BUF-HRP | |
| Chrom | TMB | |
| Subst.buffer | H2O2 | |
| STOP | SOLN | |
| WASH | SOLN | CONC |
1. Strips :12x8 (break apart) strips, 96 wells
Wells coated with anti-Rabbit IgG coated wells
 2. Anti-Estradiol: 1 vial – 6ml blue-Add 6 ml of distilled water
 3. Standards 1 to 5 + 2 controls
7 vials, 0.5 ml, lyophilised –yellow+ silver - add 0.5 ml distilled water.
See exact values on the vial labels (human serum + preservatives)
 4. Standard 0: 1 vial, 1 ml, lyophilised -yellow- add 4 ml distilled water.
0 ng/ml (human serum + preservatives)
 5. Concentrate Conjugate: 1 vial, 0.5 ml. Estradiol-HRP conjugate in phosphate
Buffer with preservatives. Pipette 0.1 ml into 1 vial of conjugate buffer
 6. conjugate buffer : 3 vials, 6 ml, red, ready to use. For dilution of
conjugate
 7. Chromogen TMB: tetramethylbenzidine ; 1 vial
1 ml green, pipette 0.2 ml into 1 vial of substrate buffer
 8. Substrate buffer: H₂O₂ in acetate/citrate buffer, 3 vials
White, ready to use, for the dilution of chromogen
 9. Stop: 1 vial, 6 ml, black, ready to use
contains 1.8 M H₂SO₄
Avoid contact with the stop solution. It may cause skin irritations and burns.
 10. Wash buffer : 1 vial, 10 ml, brown, dilute 2 ml in 400 ml dist.water
or the vial content in 2000 ml dist.water.

Note: Zero Calibrator is recommended for Samples dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. High quality distilled water
2. Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 500 µl and 4 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Horizontal microtiterplate shaker capable of 700 rpm ± 100 rpm
6. Washer for Microtiterplates
7. Microtiterplate reader capable of reading at 450 nm, 490 nm and 650 nm (in case of polychromatic reading) or capable of reading at 450 nm and 650 nm (monochromatic reading)

VII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Store the kit at 2-8°C until the exp.date.
- Once opened, store the concentrated estradiol –HRP conjugate vial at 2-8°C. Stability of diluted Estradiol conjugate is 4 hours at RT or 24h at 2-8°C. Avoid direct exposure to sunlight.
- After reconstitution, store standard and controls at 2-8°C for 1 week. For prolonged storage, these reagent have to be frozen.
- Unused strips wells are stored at 2-8°C in closed bag containing the dessicant until the exp.date.
- The concentrate washing solution is stable at RT until the exp.date.
- The freshly prepared substrate solution is stable at RT during maximum 15 minutes and must be discarded afterwards.

VIII. SPECIMEN COLLECTION AND PREPARATION

- Serum and plasma must be kept at 2 - 8 °C.
- If the test is not run within 24 hours, storage in aliquots at -20 °C is recommended. Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
- Do not use haemolysed samples.
- Do not use lipemic samples.

IX. REAGENTS PREPARATION :

Standards and controls :

Dilute Zero standard with 4 ml of distilled water and Other standard with 0.5 ml of distilled water.

HRP-Estradiol conjugate :

Pipet 0.1 ml of concentrated HRP-conjugate into one vial of conjugate buffer

Stability : 24 hours at 2-8 °C avoiding sunlight.

Washing solution :

Dilute 2 ml in 400 ml or the contents of the concentrate washing solution in 2000 ml of distilled water.

Revelation solution :

Pipet 0.2 ml of the chromogen TMB into one of the vials of substrate buffer (H₂O₂). Extemporaneous preparation is mandatory. Maximum stability before use : 15 min. at RT avoiding direct sunlight exposure.

X. PROCEDURE

A. Handling notes

- Do not use the kit or components beyond expiry date.
- Do not mix materials from different kit lots.
- Bring all the reagents to room temperature prior to use.
- Thoroughly mix all reagents and samples by gentle agitation or swirling.
- Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.
- Use a clean plastic container to prepare the Wash Solution.
- In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
- For the dispensing of the Chromogenic Solution and the Stop Solution avoid pipettes with metal parts.
- High precision pipettes or automated pipetting equipment will improve the precision.
- Respect the incubation times.
- Prepare a calibration curve for each run, do not use data from previous runs.
- The chromogenic solution should be colourless. If a dark blue colour develops within a few minutes after preparation, this indicates that the preparation is unusable and must be discarded.
- Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiterplate.
- During incubation with Chromogenic Solution, avoid direct sunlight on the microtiterplate.

B. Procedure

- . Select the required number of strips for the run.
- . The unused strips should be resealed in the bag with a desiccant and stored at 2-8 °C.
- . Secure the strips into the holding frame.

1. Add 50 µL of standards , controls and samples to designated wells.
2. Add 50 µL of conjugate to each well.
3. Add 50µl of anti-estradiol to each well.
4. Incubate for 2 hours at room temperature on an orbital shaker (600-800 rpm)
5. Aspirate and wash 4 times with 0.4 mL/well of Wash Solution.
6. Add 200 µL of Stabilized Chromogen to each well.
7. Incubate 30 minutes at room temperature with shaking.
8. Add 50 µL of Stop Solution to each well.
9. Read absorbance at 450 nm.

XI. CALCULATION OF RESULTS

Bichromatic Reading

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each calibrator against the corresponding concentration of Estradiol (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Contents (pg/ml)	OD Units	B/Bo x 100
Std 0	0.00	1.790
Std 1	13	1.424
Std 2	50	1.013
Std 3	100	0.762
Std 4	270	0.447
Std 5	935	0.221

XIII. PERFORMANCE AND LIMITATIONS

A. Detection Limit

Twenty zero standards were assayed along with a set of other standards.

The detection limit, defined as the apparent concentration two standard deviations above the average OD at zero binding, was 5 pg/ ml. (± 2)

B. Specificity

Substances	Cross-reactivity (%)
17 β Estradiol	100
Estrone	2
Estriol	1.9
E2-3-Glucuronide	0.6
E2-17-Glucuronide	0.56
E2-17-Valerate	0.1
Cortisol	<0.001
Progesterone	0.03
DHEA-Sulfate	<0.0001
Testosterone	<0.001
Androstenediol	<0.001
Norgestrel	0.01
Premarin	0.06
equilin	0.1

The % of cross-reaction was estimated under physiological conditions in serum by comparison of the concentration yielding a 50% binding inhibition

C. Precision

Intra-Assay				Inter-Assay			
Serum	n	<X> \pm SD (pg/ml)	CV (%)	Serum	n	<X> \pm SD (pg/ml)	CV (%)
A	20	131 \pm 6	4.6	C	15	101 \pm 6	6.0
B	20	257 \pm 10	3.9	D	15	196 \pm 12	6.1

D. Accuracy :

Recovery				Dilution			
Sample	Added (pg/ml)	recovered (pg/ml)	recovery (%)	dilution	Theoretical (pg/ml)	Measured (pg/ml)	recovery (%)
serum	916	719	78.4	1/1	997	997	100
	516	430	83.3	1/2	498	485	97
	316	304	96.2	1/4	249	252	101
	166	176	106.0	1/8	125	109	87

XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplo results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

XV. REFERENCE INTERVALS

Identification	Number of subjects	Range (pg/ml)
Males	50	10-45
Postmenopausal females	30	10-45
Ovulating females:	14	
Day -10		13-80
-4		20-165
-1		73-410
0 (LH peak)		118-417
+1		22-154
+4		44-174
+10		13-146
Pregnant women:		
1 st trimester	88	40-3100
2 nd trimester	39	1600-14000
3 rd trimester	100	4200-32000

Elaboratory should establish its own normal range of values.

XVI. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals.

Bovine components originate from countries where BSE has not been reported.

Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with all reagents, Stop Solution contains HCl, the chromogen contains TMB and H₂O₂. In case of contact, wash thoroughly with water.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XVII. BIBLIOGRAPHY

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XVIII. SUMMARY OF THE PROTOCOL

	CALIBRATORS(μ l)	SAMPLE(S)CONTROLS(μ l)
Calibrators (0-5)	50	-
Controls, Samples	-	50
Anti-Calci-HRP conjugate	50	50

Incubate for 2 hours at room temperature on an orbital shaker (600-800 rpm)

Aspirate the contents of each well.

Wash 4 times with 400 μ l of Wash Solution and aspirate.

Chromogenic Solution	200	200
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Incubate for 30 min at room temperature with continuous shaking at 700 rpm.

Stop Solution	200	200
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Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm) and 490 nm (versus 630 or 650 nm)

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