Total Estrogens ELISA Assay Kit

Catalog Number:
ESG31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 7.1 (22 FEB 24)

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences Total Estrogens ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Total Estrogens in human serum. The Eagle Biosciences Total Estrogens ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Total estrogens comprise the total quantity of estrone, estradiol, and estriol. 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 is on the growth and function of the reproductive tract to prepare it for the fertilized ovum. During the follicular phase of the menstrual cycle the total estrogens level shows a slight increase. The production of total estrogens 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, the production of total estrogens decreases.

In post-menopausal women the concentration of all estrogens decreases substantially and estrone becomes the predominant estrogen. In pregnant women the concentration of all estrogens escalates and estriol becomes the predominant estrogen.

A total estrogens test is commonly indicated to:

- Aid in diagnosis of sex steroid metabolism related conditions, for example, premature or delayed puberty, and aromatase and 17 alpha-hydroxylase deficiencies.
- Assess fracture risk in postmenopausal women and, to a lesser degree, older men.
- Follow-up female hormone replacement therapy in postmenopausal women.
- Prognose antiestrogen therapy, for example, aromatase inhibitor therapy.

PRINCIPLE OF THE ASSAY

The total estrogens ELISA is a competitive immunoassay. Competition occurs between total estrogens (estrone, estradiol, and estriol) present in standards, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limiting number of anti-estrogen antibody binding sites on the microplate wells. After a washing step that removes unbound materials the enzyme substrate is added and approximately 15–20 minutes later the enzymatic reaction is terminated by addition of stopping solution. The resulting optical density (OD), measured with a microplate reader, is inversely proportional to the concentration of total estrogens in the sample. A standard curve is plotted with a provided set of standards to calculate directly the concentration of total estrogens in patient samples and controls.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is intended for research use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    - Do not pipette by mouth.
    - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
    - Wear protective clothing and disposable gloves.
    - Wash hands thoroughly after performing the test.
    - Avoid contact with the eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

4. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
5. Use deionized or distilled water to dilute wash buffer concentrate.
6. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
7. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
8. A calibrator curve must be established for every run.
9. The controls should be included in every run and fall within established confidence limits.
10. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
12. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
15. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
16. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of total estrogens in human serum. The kit is not calibrated for the determination of total estrogens in other species or in specimens other than serum.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only Calibrator A may be used to dilute any high serum samples. The use of any other reagent (including water) will lead to false results.
5. This kit is intended for research use only and should not be used as a diagnostic tool.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

No specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Single-channel pipette to dispense 50 µL
2. Multi-channel pipettes to dispense 50 µL and 150 µL
3. Multi-channel pipettes to dispense 350 µL (if washing manually)
4. Disposable pipette tips
5. Distilled or deionized water
6. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)
7. Microplate washer

REAGENTS PROVIDED

1. **Rabbit Anti-Estrogens Antibody-Coated Break-Apart Well Microplate** — Ready To Use
   - Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

2. **Estrogen-HRP Conjugate** — Ready to Use
   - Contents: Estrogen-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 20 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

3. **Calibrators** — Ready To Use
   - Contents: Seven vials containing estrogen in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of estrogens.

   * Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
A	0 pg/mL	2.0 mL
B	25 pg/mL	1.0 mL
C	50 pg/mL	1.0 mL

D	100 pg/mL	1.0 mL
E	250 pg/mL	1.0 mL
F	500 pg/mL	1.0 mL
G	1000 pg/mL	1.0 mL
H	2500 pg/mL	1.0 mL

Storage: Refrigerate at 2–8°C.
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

4. **Controls** — Ready to Use
   Contents: Two vials containing estrogen in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of total estrogens. Refer to vial labels for the acceptable range. The concentration of the controls was verified by a second party with a CDC HoSt certified method.
   Volume: 1.0 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

5. **Wash Buffer Concentrate** — Requires Preparation x10
   Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   Volume: 50 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450mL of distilled or deionized water
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
   Working wash buffer stability: Following preparation, the working wash buffer is stable for up to 2 weeks when stored at 2-8C.

6. **TMB Substrate** — Ready To Use
   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   Volume: 16 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

7. **Stopping Solution** — Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

ASSAY PROCEDURE

Specimen Pretreatment: None

Bring reagents, samples, and the microplate to room temperature before use. Test the calibrators, controls and specimen samples in duplicate. Once the procedure has started, complete all steps without interruption.

1. Prepare the working solution of wash buffer.
2. Remove the required number of well strips from the microplate. Reseal the bag and return any unused strips to the refrigerator.
3. **Pipette 50 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. **Incubate** the microplate on a microplate shaker** for 30 minutes at room temperature.
5. **Pipette 150 µL** of the Estrogen-HRP conjugate into each well. (We recommend using a multichannel pipette.)
6. **Incubate** for 2 hours at room temperature (no shaking). Cover the plate to avoid any contamination.
7. **Wash** the wells 3 times with 350 µL of diluted wash buffer per well. Tap the plate firmly against absorbent paper to ensure that no droplets remain in the wells. The use of a microplate washer is recommended. If a washer is not available, ensure the wash buffer reaches the top edge of the wells and no liquid remains in the plate after the final washing.)
8. **Pipette 150 µL** of the TMB substrate into each well at timed intervals.
9. **Incubate** for 30 minutes at room temperature (or until calibrator A attains dark blue color).
10. **Pipette 50 µL** of stopping solution into each well at the same timed intervals as in step 8. Gently tap the side of the microplate to mix the contents of the wells.
11. **Read** the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Use a 4-parameter curve fit with immunoassay software to generate concentration results.
3. If no software is available draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 10,000 pg/mL then dilute it with calibrator A at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

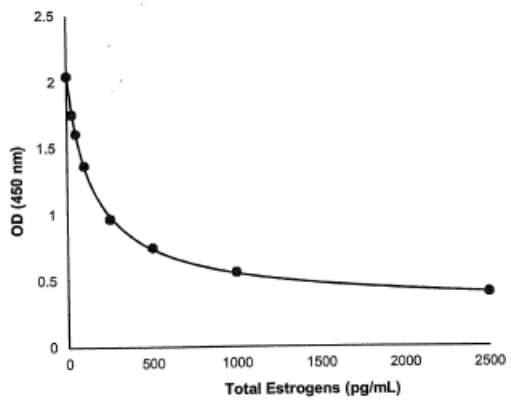
1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable ranges as stated in the QC Certificate. % Binding = (OD of calibrator / OD of calibrator A) x 100.
3. The values obtained for the kit controls are withing the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.044	100	0
B	1.755	86	25
C	1.609	79	50
D	1.368	67	100
E	0.964	47	250
F	0.744	36	500
G	0.561	27	1000
H	0.407	20	2500
Unknown	7.91	-	400

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS
SPECIFICITY (CROSS-REACTIVITY)

The cross-reactivity was evaluated in relation to estrogens reacting at 100%.

Steroid	% Cross Reactivity
Estrone	100
17β-Estradiol	100
Estriol	100
11-Deoxycorticosterone	0.4
17-Hydroxyprogesterone	0.3
17a-Estradiol	5.3
Aldosterone	0.2
Androstenedione	< 0.01
Cholesterol	0
Corticosterone	< 0.01
Cortisol	<0.1
DHEA	0.3
DHEAS	0.004
DHT	0.5
Equilin	6.3
Estradoil Sulfate	0.1
Estrone Sulfate	0.07
Prednisone	0
Pregnenolone	<0.1
Pregnenolone	<0.1
Progesterone	<0.1
Testosterone	0.3

INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 10 mg/dL, Triglycerides up to 5 mg/mL, Biotin up to 2.4 µg/mL, Daidzein and Resveratrol up to 200 ng/mL, Genistein up to 100 ng/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1.2 IU/mL did not interfere with the assay.

Note on Fulvestran

Estradiol immunoassays have been reported to show interference from the drug Fulvestran (Faslodex®). This cross-reactivity can cause falsely elevated estrogen levels in patients under Fulvestran treatment. The following results were obtained with the Total Estrogens ELISA kit after pooled serum samples from three cohorts were spiked to a concentration of 25 ng/mL of Fulvestran.

Sample	Unspiked Sample (pg/mL)	Sample Spiked to 25 ng/mL Fulvestran (pg/mL)
Pool 1	106.8	128.6
Pool 2	87.8	105.8
Pool 3	326.4	377.6

The Cmax has been reported as 11.4 ng/mL (Robertson and Harrison, 2004) and 25.1 ng/mL (AstraZeneca Canada, 2017)

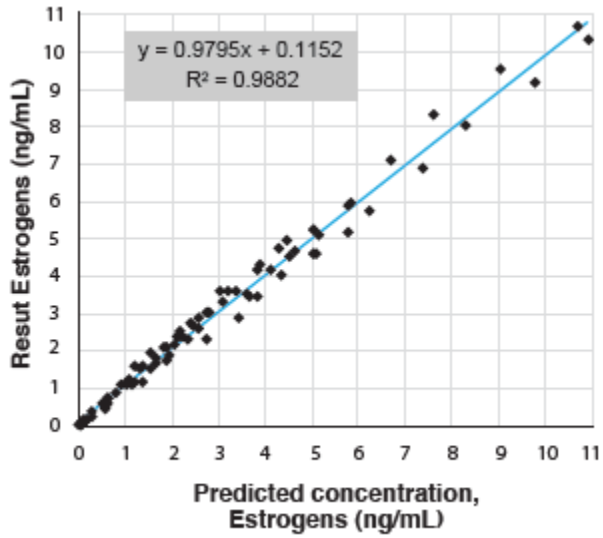
PRECISION

Spiked samples were prepared by adding defined amounts of estrone to three patient serum samples. The results (in pg/mL) are tabulated below:

Sample	Mean	Within Run SD	Within Run CV	Between Run SD	Between Run CV	Total SD	Total CV
1	104.6	6.6	6.3%	8.3	8.0%	11.9	11.4%
2	56.5	5.3	9.3%	7.0	12.4%	8.8	15.5%
3	377.2	17.6	4.7%	10.8	2.9%	24.4	6.5%
4	83.3	4.7	5.7%	4.2	5.0%	7.1	8.5%
5	100.2	6.0	6.0%	7.5	7.4%	9.9	9.9%
6	251.8	10.3	4.1%	13.3	5.3%	17.0	6.8%
7	365.9	16.8	4.6%	52.2	14.3%	54.8	15.0%
8	1276.7	78.9	6.2%	46.8	3.7%	98.0	7.7%

LINEARITY

The linearity study was performed with four human serum samples covering the range of the assay and following CLSI guideline EP06-A. The samples were diluted in calibrator A at several equidistant concentration levels and up to 10 percent (1:10), tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using calibrator A as the diluent.



COMPARATIVE STUDIES

The DBC Total Estrogens ELISA kit (y) was compared to Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS (x). The comparison of 45 serum samples (between 20 and 4300 pg/mL) yielded the following linear regression results:

y = 0.89x – 62, r = 0.99

REFERENCE RANGES

Reference ranges were obtained from individuals from diverse races and without regard of menopausal status. Each laboratory shall establish their own range of reference values.

Group	N	Median (pg/mL)	95% Confidence Range (pg/mL)
Pre-Menopausal Females			
1-10 Days	40	120	16-328
11-20 Days	40	136	34-501
21-30 Days	40	168	48-350
Post Menopausal Females	120	74	40-244
Adult Males	120	104	56-213

References

1. Faslodex® Product Monograph. AstraZeneca Canada, 2017
2. Robertson JFR and Harrison M. Fulvestran Pharmacokinetics and pharmacology. British Journal of Cancer. 2004; 90:S7-S10.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident. Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.