Estradiol ELISA Kit

Catalog Number: ESD31-K01

For Research Use Only. Not for use in diagnostic procedures.

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INTENDED USE

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

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

INTRODUCTION

Estradiol is one of the main components of naturally occurring estrogens and is the major estrogen secreted during the menstrual cycle. The serum levels of estradiol are low during the follicular phase rising gradually until about one day before ovulation when a marked rise in the estradiol level occurs (Ovulatory Peak). The estradiol level falls rapidly at, or right after ovulation and is again within the levels of the follicular phase. There is a second rise of estradiol around day 21 of the cycle (Luteal Peak). The levels then decline gradually to the lowest level at the onset of the next menstrual cycle.

PRINCIPLE OF THE ASSAY

The Estradiol ELISA Assay Kit is a competitive immunoassay. Competition occurs between an estradiol present in calibrators, controls and specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of antibody binding sites on the microplate wells. After a washing step that remove unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of estradiol present. Following an incubation, the enzymatic reaction is terminated by addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate plate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of estradiol in specimen samples and controls can be directly read.

LIMITATIONS RELATED TO INTENDED USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing
3. The kit is calibrated for the determination of estradiol in human serum. The kit is not calibrated for the determination of estradiol in other specimens of human or animal origin.
4. The results obtained with this kit shall not be used for a clinical diagnosis and for therapeutic decisions.
5. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.
6. This assay should not be used for specimens of individuals being treated with the drug fulvestrant (Faslodex®) which cross reacts with estradiol and could lead to a falsely elevated test result.

WARNINGS AND PRECAUTIONS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
    a. Do not pipette by mouth.
    b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.

c. Wear protective clothing and disposable gloves. d Wash hands thoroughly after performing the test. e Avoid contact with eyes, use safety glasses; in case of contact with 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. Do not use this kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or saliva pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >3200 pg/mL. If further dilution and retesting is required, only Calibrator A may be used to dilute saliva samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type

and speed of saker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
30. 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.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS
The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens, All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS
Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage
Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°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 Pre-Treatment
This assay is a direct system; no specimen pretreatment is necessary.

MATERIALS NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 50 µL.
2. Calibrated multi-channel pipette to dispense 50 µL, 100 µL, and 150 µL.
3. Calibrated multi-channel pipettes to dispense 300 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker:
    a. Orbital shaker (3 mm diameter) set to 600 rpm or
    b. Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.

REAGENTS PROVIDED

1. **MICROPLATE**

Contents:	One anti-estradiol polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

2. **HRP Conjugate**

Contents:	One bottle containing Estradiol-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative
Format:	Concentrated; Requires Preparation
Volume:	0.3 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.
Preparation of HRP Conjugate Working Solution:	**Dilute 1:51** in assay buffer before use (e.g., 40 µL of conjugate concentration in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over

3. **Calibrator A – F**

Contents:	Six bottles of calibrator containing specified estradiol concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with define quantities of estradiol. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 20, 100, 300, 800, 3200 pg/mL
Format	Ready to Use
Volume	Calibrator A 2.0 mL/bottle Calibrator B – F: 0.5 mL/bottle
Storage	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

4. **Control 1 – 2**

Contents:	Two bottles of control containing different estradiol concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of estradiol. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use

Volume:	0.5 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

5. **Assay Buffer**

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative
Format:	Ready to Use
Volume:	15 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

6. **TMB Substrate**

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	16 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

7. **Stopping Solution**

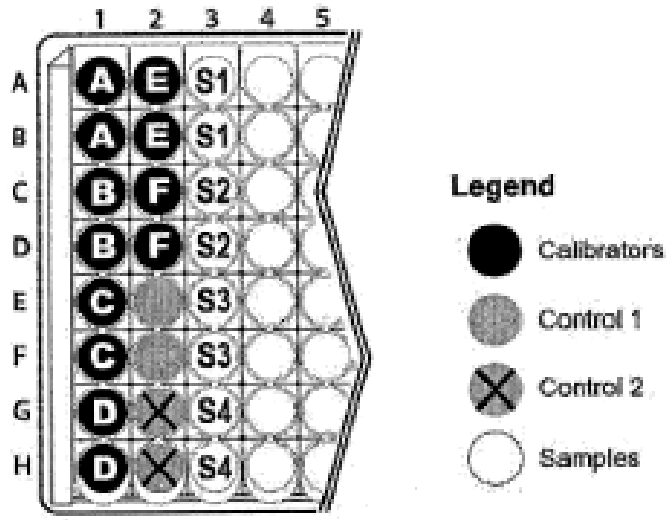
Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 mL/bottle
Storage:	2-8°C
Stability	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.
Safety	Refer to product SDS.

8. **Wash Buffer Concentrate**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until expiry date printed on the label. After Opening: Stable for two weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.

Preparation of Wash Buffer Working Solution:	**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 450 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Specimen Pretreatment: None

All kit components, controls, and specimen samples must reach room temperature prior to use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, **mix** gently by inversion.
2. **Prepare** the HRP Conjugate Working Solution and Wash Buffer Working Solution (See section *Reagents Provided, HRP Conjugate Concentration, Wash Buffer Concentrate*).
3. **Plan** the microplate wells to be used for calibrators, controls, and samples. See *Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. **Pipette 50 µL** of each calibrator, control, and pre-treated specimen sample into assigned wells.
5. **Pipette 100 µL** of the HRP conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
6. **Incubate** the microplate on a microplate shaker** for **60 minutes** at room temperature.
7. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as state below.
    a. **Automatic:** Using an automatic microplate washer, perform a **3-cycle** wash using **300 µL /well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of Wash Buffer Working Solution. After the final

wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

b. **Manually:** For manual washing, perform a **3-cycle** wash using **300 µL /well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waster container, then pipetting 300 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

8. **Pipette 150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
9. **Incubate** the microplate on a microplate shaker** for **10-15 minutes** at room temperature.
10. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for the addition of TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
11. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

******See Section *Reagents and Equipment Needed But Not Provided* for microplate shaker options

CALCULATIONS

1. Calculate the mean optical density of each calibrator, control, and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 3200 pg/mL and needs to be diluted and retested, then dilute it with calibrator A not more than 1:8. 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:

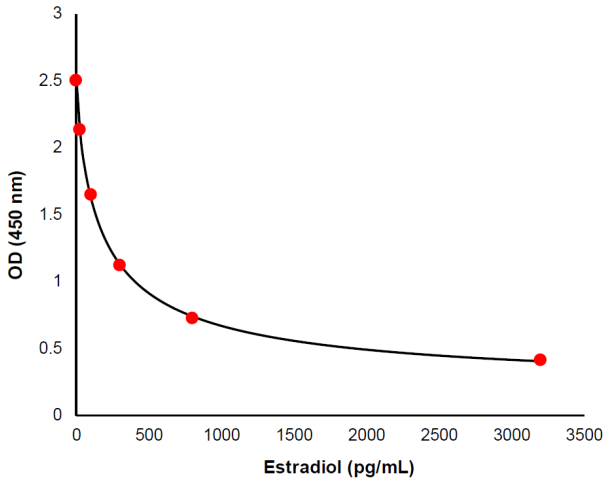
1. The Calibrator A mean optical density meets the acceptable range as stated in the QC Certificate
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator / OD of calibrator A) X 100.
3. The values obtained for the kit controls are within 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	% Binding	Value (pg/mL)
A	2.505	100	0
B	2.140	85	20
C	1.651	66	100
D	1.122	45	300
E	0.732	29	800
F	0.417	17	3200
Unknown	1.773	-	69.6

TYPICAL CALIBRATOR CURVE



PERFORMANCE AND CHARACTERISTICS

Sensitivity

The detection limit is defined as the concentration of estradiol needed to give a B/B0 values equivalent to the point where B is equal to B0 minus 2X the SD of B0. Based on 20 replicate analyses of standard A, the sensitivity is 10 pg/mL.

Specificity (Cross-Reactivity)

The following compounds were tested for cross-reactivity with the Direct Estradiol ELISA kit with estradiol cross-reacting at 100%.

Steroid	**% Cross Reactivity**
Estradiol	100
Estriol	1.6
Estrone	1.3
Progesterone	0.1
Cortisol	0.1

This assay should not be used for patients being treated with the drug fulvestrant (Faslodex®) which cross reacts with estradiol and could lead to a falsely elevated test result.

Intra-Assay Precision

Three samples were assayed ten times each on the same calibrator curve. The results (in pg/mL) are tabulated below:

Sample	M ean	SD	CV %
1	85.624	7.946	9.3
2	355.735	32.372	9.1
3	1104.385	51.243	4.6

Inter-Assay Precision

Three samples were assayed ten times over a period of four weeks. The results (in pg/mL) are tabulated below:

Sample	Mean	SD	CV %
1	82.044	8.286	10.1
2	324.623	31.813	9.8
3	1153.301	71.505	6.2

Recovery

Three human serum samples were spiked with defined amounts of estradiol. The recovery results (in pg/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	43.312	-	-
+800 (20%)	196.874	169.427	116.2
+3200 (10%)	360.670	330.284	109.2
+3200 (20%)	638.328	569.427	112.1
2 Unspiked	125.661	-	-
+800 (20%)	275.461	238.051	115.7
+3200 (10%)	415.680	405.146	102.6
+3200 (20%)	576.160	638.051	90.3
3 Unspiked	336.297	-	-
+800 (20%)	474.791	413.581	114.8
+3200 (10%)	600.214	596.634	100.6
+3200 (20%)	758.257	813.581	93.2

Linearity

Three human serum samples were diluted with calibrator A. The linearity results (in pg/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	638.328	-	-
1:2	272.247	319.164	85.3
1:4	140.592	159.582	88.1
1:8	74.844	79.791	93.8
2	576.160	-	-
1:2	324.092	288.080	112.5
1:4	168.957	144.040	117.3
1:8	73.646	72.020	102.3
3	758.257	-	-
1:2	335.908	379.129	88.6
1:4	186.152	189.564	98.2
1:8	78.103	94.782	82.4

REFERENCE RANGES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. The results of an expected range study with apparently normal healthy subjects yielded the following results (all values are reported in pg/mL):

Group	N	Mean	Central 95%
Males	40	22	<100
Females			
Follicular Phase	10	41	15-120
Ovulation	3	289	200 - 400
Lutueal Phase	10	193	175 - 325
Postmenopausal	30	28	<90

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WARRANTY INFORMATION

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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.