BioVendor Group

ENG

Product Data Sheet:

ESTRADIOL ELISA

Catalogue number:

RCD011R

For research use only!

BioVendor R&D

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CONTENTS

1. INTENDED USE

For the quantitative measurement of estradiol in human serum by an ELISA (enzyme-linked immunosorbent assay).

This kit is intended for professional use only and is for laboratory use only.

For reserch use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

2. PRINCIPLE OF THE TEST

The Estradiol ELISA is a competitive immunoassay. Competition occurs between estradiol present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of antiestradiol antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of estradiol present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate 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.

3. CLINICAL APPLICATIONS

17β-Estradiol, or Estradiol, is predominantly produced by the ovaries in premenopausal females (1, 2), testes in males (3), and in adipose tissue (4) and the adrenal glands (5) of males and females. Males and postmenopausal females have the lowest estradiol concentration levels (6). In non-pregnant, premenopausal females, estradiol concentrations fluctuate during the menstrual cycle with a peak just before ovulation (7). Estradiol remains elevated during the luteal phase, before decreasing to lower concentrations during the follicular phase.

4. PROCEDURAL CAUTIONS AND WARNINGS

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: • 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 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 the 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 serum 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 procedural techniques or pipetting, incomplete washing, or 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 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.
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 >4,000 pg/mL. If further dilution and retesting is required, only a serum sample with a low concentration of estradiol (< 40 pg/mL) may be used to dilute serum 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 the 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 shaker used can influence the optical densities and test results. If a different type of shaker 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 patient is established.

5. LIMITATIONS

1. This test is not to be used for cancer diagnostics.
2. This test is not intended to be used for screening purposes.
3. This test is not intended for home testing or self-testing.
4. 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.
5. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
6. 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.
7. 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.

6. PROCEDURAL CAUTIONS AND WARNINGS

6.1 Biohazard

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

6.2 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.

7. SPECIMEN COLLECTION AND STORAGE

Approximately 0.15 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 analysis is to be performed at a later date. Specimens may be more stable than indicated.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

8. SPECIMEN PRETREATMENT

Specimen pre-treatment is not required.

9. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 50 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL, 100 µL and 150 µL.
3. Calibrated multi-channel pipettes to dispense 350 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.

10. REAGENTS PROVIDED

10.1 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.

10.2 HRP Conjugate

Contents: One bottle containing Estradiol-Horse Radish Peroxidase (HRP) conjugate in 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.

10.3 Calibrator A – G

Contents: Seven bottles of calibrator containing specified estradiol concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of estradiol.
Listed below are approximate concentrations, please refer to vial labels for exact concentrations.
Concentrations: 0, 25, 100, 400, 1000, 2000, 4000 pg/mL.

Format: Ready to Use
Volume: Calibrator A-G: 1.0 mL/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

10.4 Controls

Contents: Two bottles of control containing different estradiol concentrations. Protein-based buffer with a nonmercury 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: 1.0 mL/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for two weeks.

10.5 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.

10.6 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.

10.7 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 the 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.

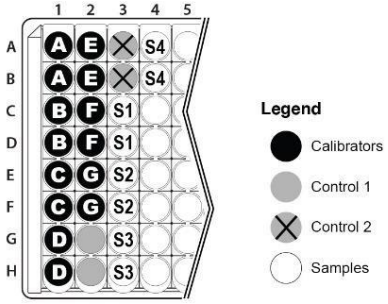
Preparation of Wash Buffer Working Solution:

X10

Dilute 1:10 Before Use

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.

11. RECOMMENDED ASSAY LAYOUT



12. 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 Wash Buffer Working Solution (See section 9. Reagents Provided, 7. Wash Buffer Concentrate).
3. Plan the microplate wells to be used for calibrators, controls, and samples. See section 10. 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 specimen sample into assigned wells.
5. Pipette 100 µL of the HRP Conjugate 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 stated below.

   Automatic: Using an automatic microplate washer, perform a 3cycle wash using 350 µL/well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 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.

Manually: For manual washing, perform a 3-cycle wash using 350 µL/well of Wash Buffer Working Solution (3 x 350 µL).

One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waster container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multichannel 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 multichannel pipette is recommended).
9. Incubate the microplate on a microplate shaker** for 30 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 the 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 the addition of the Stopping Solution.

** See section 8. Reagents And Equipment Needed But Not Provided for microplate shaker options.

13. CALCULATIONS

1. Calculate the mean optical density for 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 4,000 pg/mL and needs to be diluted and retested, then dilute the sample with a sample containing a low concentration of estradiol (<40 pg/mL) up to a 1:8 dilution. The result obtained must be multiplied by the dilution factor.

14. QUALITY CONTROL

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

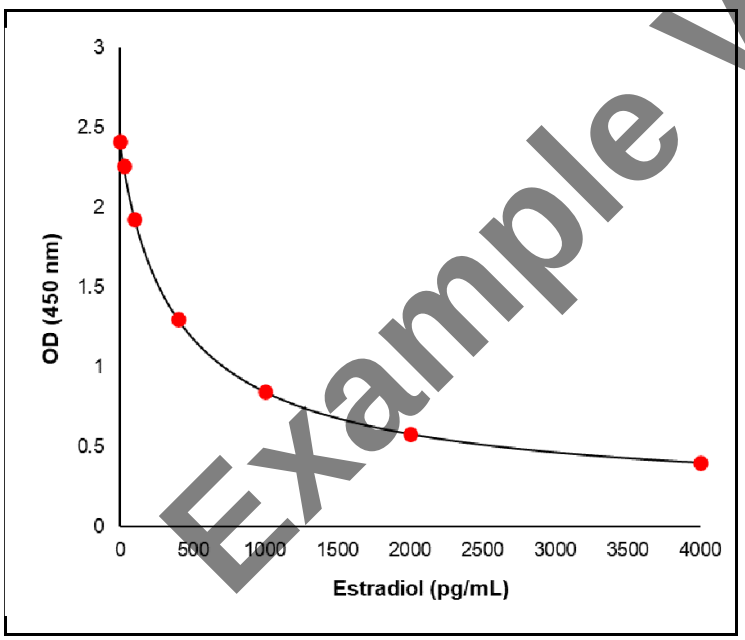
1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator/OD of calibrator A) x 100.
3. The values obtained for 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.

15. TYPICAL TABULATED DATA

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.413	100	0
B	2.261	94	25
C	1.927	80	100
D	1.299	54	400
E	0.848	35	1000
F	0.581	24	2000
G	0.403	17	4000
Unknown	1.617	-	211.3

16. TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



17. PERFORMANCE CHARACTERISTICS

17.1 Sensitivity

The Limit of Detection (LoD) was determined by testing 20 replicates of Calibrator A in a single test, with kit calibrators and quality controls run in duplicate. The LoD was determined as the concentration corresponding to the mean OD450 of calibrator A minus 2 x the standard deviation of the 20 replicates. The resulting OD450 was converted into a concentration value using microplate reader data analysis software.

The LoD was determined to be 8.4 pg/mL

17.2 Specificity (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with 17β-estradiol cross-reacting at 100%.

Compound	% Cross-Reactivity
17β-Estradiol	100
Cortisol	<0.00025
Estrone	2.0
Estriol	1.9
Progesterone	<0.025

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.

17.3 Precision

Intra-Assay Precision

Three human serum samples with low, moderate and high concentrations of estradiol were assayed ten times on a single curve. The results (in pg/mL) are summarized in the table below:

Sample	Mean	SD	CV%
1	80.550	7.765	9.639
2	278.177	15.189	5.460
3	1600.247	50.783	3.163

Inter-Assay

Three human serum samples with low, moderate and high concentrations of estradiol were tested in duplicate in ten different assays using a single lot of the kit. The results (in pg/mL) are summarized in the table below:

Sample	Mean	SD	CV%
1	90.644	10.974	12.1
2	300.300	21.545	7.17
3	1647.595	62.113	3.77

Lot-to-lot Reproducibility

A lot-to-lot reproducibility study was performed using two different kit lots of the Estradiol ELISA following CLSI guideline EP26-A. A total of 360 serum samples were run in singlicate with both kit lots. The comparison between lots yielded the following linear regression results:
y = 1.053x – 7.213, r = 0.99

17.4 Linearity

Three human serum samples were diluted with a low value estradiol serum sample (< 40 pg/mL) at several equidistant concentration levels and up to a 1:8 dilution. Samples were tested in duplicate, and the results compared to the predicted concentrations. The results (in pg/mL) are tabulated below.

Sample	Obs.Result	Exp.Result	Recovery%
1	2452.7	-	-
1:2	1273.8	1226.4	103.9
1:4	610.9	613.2	99.6
1:8	323.9	306.6	105.6
2	803.8	-	-
1:2	438.2	401.9	109.0
1:4	213.8	201.0	106.4
1:8	118.1	100.5	117.5
3	476.3	-	-
1:2	255.1	238.2	107.1
1:4	121.2	119.1	101.8
1:8	73.4	59.5	123.4

17.5 Recovery

Three human serum samples were spiked (1:9) to three different concentration levels of estradiol. The original unspiked samples and each set of spiking samples were tested in duplicate. Expected concentration values were determined by the fraction contribution of each sample and spiking sample solution to the final mix. The recovery (%) was calculated as the ratio-percent between the sample result and expected value. The results (in pg/mL) are tabulated below:

Sample	Spike Sample Concentration	Observed Result	Expected Result	Recovery %
1 Unspiked	-	298.2	-	-
	4006	551.2	669.0	82.4
	3280	512.0	596.4	85.9
	2138	423.4	482.2	87.8
2 Unspiked	-	117.2	-	-
	4006	394.8	506.1	78.0
	3280	356.4	433.5	82.2
	2138	260.6	319.3	81.6
3 Unspiked	-	78.6	-	-
	3804	405.2	451.2	89.8
	3119	363.2	382.7	94.9
	1924	230.9	263.2	87.7

17.6. Comparative studies

The DBC Estradiol ELISA kit (y) was compared against a Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) method (x). The comparison of 104 serum samples yielded the following linear regression results:

$y = 1.44x + 49.24$, $r = 0.97$

18. REFERENCE RANGES

Reference ranges (95%) were estimated following CLSI guideline EP28-A3c. Human serum samples were from putatively healthy adults of diverse races. The table below summarizes the results. Each laboratory shall establish their own reference ranges

Group	N	Median (pg/mL)	95% Confidence Range (pg/mL)
Males	120	66.0	6.2 – 224.8
Premenopausal Females: menstrual cycle day 1 – 10	40	90.5	27.6 – 311.7
Premenopausal Females: menstrual cycle day 11 – 21	40	113.1	15.4 – 360.6
Premenopausal Females: menstrual cycle day 21 – 30	40	144.0	9.6 – 270.2
Postmenopausal Females	120	38.8	ND – 287.1

ND = Not Detectable; lower than the LoD

19. REFERENCES

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