Estradiol ELISA

For the quantitative determination of Estradiol in human serum.

For "In Vitro Diagnostic" use within the United States of America.
This product is for "Research Use Only" outside of the United States of America.

Catalog Number:	11-ESTHU-E01
Size:	96 wells
Version:	6.2 August 09, 2013 ALPCO September 19, 2013

INTENDED USE

For the direct quantitative determination of Estradiol by enzyme immunoassay in human serum. For in-vitro diagnostic use.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled estradiol (present in standards, controls and samples) and an enzyme-labelled estradiol (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stop solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of unlabeled estradiol in the sample. A set of standards is used to plot a standard curve from which the amount of estradiol in samples and controls can be directly read.

PRINCIPLE OF TEST

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.

PROCEDURAL CAUTIONS AND WARNINGS

1. 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.
2. It is recommended to all customers to prepare their own control materials or serum pools that should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. 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.
6. A calibrator curve must be established for every run.
7. The controls (included in kit) should be included in every run and fall within established confidence limits.
8. 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.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) 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.
11. When dispensing the substrate and stop solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

13. 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.
14. 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 estradiol in human serum. The kit is not calibrated for the determination of estradiol in saliva, plasma or other specimens of human or animal origin.
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 may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. 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 0.2 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

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED

1. Rabbit Anti-Estradiol Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.
Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8^{o}C
Stability: 12 months or as indicated on label.

2. Estradiol-Biotin : Avidin-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.
Contents: Estradiol-Biotin and Avidin-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 300 µl/vial
Storage: Refrigerate at 2-8^{o}C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of conjugate in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of HRP in 12 ml of assay buffer. Discard any that is left over.

3. Estradiol Calibrators - Ready To Use.
Contents: Six vials containing estradiol in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of estradiol.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 pg/ml	2.0 ml
Calibrator B	20 pg/ml	0.5 ml
Calibrator C	100 pg/ml	0.5 ml
Calibrator D	300 pg/ml	0.5 ml
Calibrator E	800 pg/ml	0.5 ml
Calibrator F	3200 pg/ml	0.5 ml

Storage: Refrigerate at 2-8^{o}C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls - Ready To Use.
Contents: Two vials containing estradiol in a protein-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of estradiol. Refer to vial labels for the acceptable range.
Volume: 0.5 ml/vial
Storage: Refrigerate at 2-8^{o}C
Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Assay Buffer - Ready To Use.
Contents: One vial buffer containing a dissociate agent with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

6. Wash Buffer Concentrate - Requires Preparation.
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 plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

7. 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: 12 months or as indicated on label.

8. Stop Solution - Ready To Use.
Contents: One vial containing 1M sulfuric acid.
Volume: 6 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

ASSAY PROCEDURE
Specimen Pretreatment: *None.*

All reagents must reach room temperature before 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. Prepare working solutions of the estradiol-biotin: avidin-HRP conjugate and wash buffer.

2. Remove the required number of microwell strips. 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. Pipette 100 µl of the conjugate working solution into each well (The use of a multichannel pipette is recommended).

5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.

6. Wash the wells three times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).

7. Pipette 150 µl of TMB substrate into each well at timed intervals.

8. Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator A attains dark blue color for desired OD).

9. Pipette 50 µl of stop solution into each well at the same timed intervals as in step 7.

10. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stop solution.

*If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of the patient/control samples.

CALCULATIONS

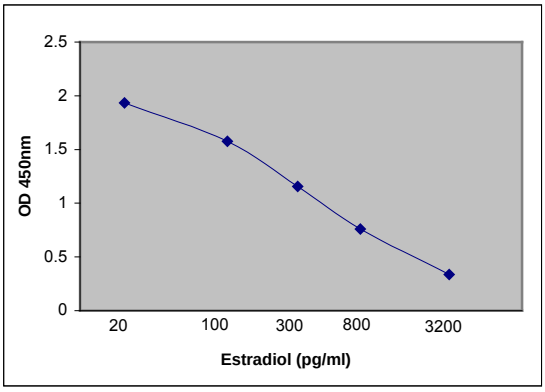
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 3200 pg/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (pg/ml)
A	2.001	1.952	1.976	0
B	1.716	1.775	1.746	20
C	1.397	1.356	1.377	100
D	0.902	0.883	0.893	300
E	0.612	0.702	0.657	800
F	0.365	0.368	0.367	3200
Unknown	1.428	1.451	1.440	77.417

TYPICAL CALIBRATOR CURVE

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



PERFORMANCE 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 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

INTRA-ASSAY PRECISION

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

Sample	Mean	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. 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
1 Unspiked	125.661	-	-
+800(20%)	275.461	238.051	98.2
+3200(10%)	415.680	405.146	102.6
+3200(20%)	576.160	638.051	90.3
2 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

EXPECTED NORMAL VALUES

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
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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