Human E2 ELISA Kit

to quantitatively determine Human E2 in Human Blood Serum or Plasma

INSTRUCTION MANUAL

FOR ELISA KIT No: LTHS802K

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INTENDED USE

This kit is used to quantitatively determine the Human E2 in the sample of Human Blood Serum or Plasma. For *in vitro* use only.

MANUAL VERSION 1.02X2

BACKGROUND

Estradiol (E2) is a steroid hormone with molecular weight 272.4 Da. In humans, E2 shows the highest physiological activity among the estrogens. In males, minor quantities of E2 are producyd by adrenals and testicles. In females, E2 is produced by ovarian follicles. The physiological activity of E2 involves multiple functions in female reproductive system. Regulation of E2 production and secretion is complex and depends on pituitary and ovarian hormones. Serum E2 level is low in follicular phase of the menstrual cycle; 3–5 days before serum LH peak, serum E2 level begins to rise and reaches a maximum ca. 12 hours before LH peak. After LH peak, E2 level drops dramatically to the minimal level and starts to rise again. The maximal E2 level in serum is observed in luteal phase, at day 9 after ovulation; then the decline of serum E2 reflects the degradation of corpus luteum. During pregnancy, the determination of serum E2 reflects the status of fetoplacental system. In first trimester, serum E2 level is in the range corresponding to the ovulation levels. Sharp increase of serum E2 in pregnant women is observed between 9th and 10th week; then the increase continues less sharply by the end of pregnancy. Increased levels of serum estradiol are characteristic for metrorrhagias in postmenopausal age; adrenal hyperplasia; estrogen-secreting tumours; liver cirrhosis; feminization in children and males; intake of gonadotropins and estrogens. Decreased levels of serum estradiol are observed in Turner syndrome, primary or secondary hypogonadism; germaphroditism; post-climacteric syndrome; fetal dysfunctions; intake of oral contraceptives.

PRINCIPLE

This test is based on competition enzyme immunoassay principle. Tested specimen

is placed into the microwells coated by specific rabbit polyclonal to estradiol-antibodies simultaneously with conjugated Estradiol-peroxidase. Estradiol from the specimen competes with the conjugated Estradiol for coating antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is inversely related to the quantity of the measured analyte in the specimen.

ASSAY RESTRICTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C. Immediately after use remaining reagents should be returned to cold storage at 4˚C

MATERIALS SUPPLIED IN THIS KIT

1. E2 Strip Plates, 8 X 12 wells. Qty: 1
2. Calibrators: C1: 0, C2: 0.1, C3: 0.3, C4: 1, C5: 3, C6:20 nmol/l, 6pcs
3. Control Serum, Qty: 1
4. Conjugate, Qty: 1
5. Substrate Solution, Qty: 1
6. Wash Buffer Concentrate 21X, Qty: 1
7. Stop Solution, Qty: 1
8. Plate Sealing Tape, Qty: 2
9. Instruction Manual, Qty: 1

PRECAUTIONS

Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer. 2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided. 3. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each

standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

• To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

• When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

• Substrate solution should remain colourless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

SAMPLE COLLECTION & STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided. Specimens may be stored for up to 48 hours at +2…+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/ thawing should be avoided. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20˚C. Avoid repeated freeze-thaw cycles. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 ˚C. If samples are to be used within 24 hours, they may be stored at 2 to 8 ˚C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

—All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.

—All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.

—It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.

—Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

—It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

— Note that for E2: 1 nmol/l = 272 pg/ml

ASSAY PROCEDURE

1 Put the desired number of microstrips into the frame; allocate 14 wells for the calibrators CAL 1–6 and control samples CONTROL and two wells for each

unknown sample.

DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.

2 Pipet 25 µl of calibrators CAL 1–6, control samples CONTROL and unknown samples into the wells.

3 Dispense 100 µl of conjugate solution into the wells. Cover the wells.

4 Incubate 60 minutes at 37 °C.

5 Prepare washing solution by 21x dilution of washing solution concentrate (BUF WASH 21X) by distilled water. Wash the strips 5 times.

6 Dispense 100 µl of SUBS TMB into the wells

7 Incubate 10–20 minutes at +18…+25 °C

8 Dispense 100 µl of STOP into the wells.

9 Measure OD (optical density) at 450 nm.

10 Set photometer blank on first calibrator

11 Apply lin-log method for data reduction.-

EXPECTED VALUES AND CHARACTERISTICS

The following normal range is recommended (see below).

NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, nmol/l		Units alternative, pg/ml	
	Lower limit	Upper limit	Lower limit	Upper limit
Children under 11 yrs	-	0.2	-	54.4
Males	0.029	0.3	7.9	81.6
Females				
Pregnancy week:				
1st trimester	0.1	10.5	27	2856
2nd trimester	3.0	21	816	5712
3rd trimester	6.0	80	9792	21760
Menstrual cycle:				
follicular phase	0.14	0.70	38.1	190.4
luteinic phase	0.17	1.10	46.2	299.2
ovulation	0.34	1.80	92.5	489.6
post menopausal	-	0.23	-	62.6

PERFORMANCE CHARACTERISTICS

Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
Estradiol	100
Estrone	0.2
Estriol	0.6
Cortisol	0.06
Prednisolone	0.09
Corticosterone	<0.01
Progesterone	<0.01
17-Hydroxyprogesterone	<0.05
Pregnenolone	<0.05
Testosterone	<0.01

Sensitivity of the assay was assessed as being 0.05 nmol/l.

Linearity was checked by assaying dilution series of 5 samples with different E2 concentrations. Linearity percentages obtained ranged within 90 to 110%.

Recovery was estimated by assaying 5 mixed samples with known E2 concentrations. The recovery percentages ranged from 90 to 110%

CALCULATION

Calculate the mean absorbance values (OD450) for each pair of calibrators and samples. Plot a calibration curve on graph paper: OD versus E2 concentration. Determine the corresponding concentration of E2 in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).