17 β-Estradiol

Enzyme immunoassay for the quantitative determination of 17 β-Estradiol in human serum or plasma

Only for in-vitro diagnostic use

Product Number: DNOV003 (96 Determinations)
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1. INTRODUCTION

Estradiol (17β-Estradiol) is a sex hormone. It represents the major Estrogen in humans. Estradiol has not only a critical impact on reproductive and sexual functioning, but also affects other organs including bone structure. During the reproductive years most Estradiol in women is produced by the ovaries, smaller amounts of Estradiol are also produced by the adrenal cortex. In men, the testes produce Estradiol. In plasma Estradiol is largely bound to sex hormone binding globulin (SHBG), also to albumin, only a fraction is free and biologically active. Serum Estradiol measurement in women reflects primarily the activity of the ovaries. During pregnancy Estrogen levels, including Estradiol, rise steadily towards term. Estradiol increases due to placental production. In adult premenopausal women, ovarian production of Estradiol is stimulated by luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) during the menstrual cycle. In adult women, Estradiol levels are measured in the evaluation of fertility and menstrual irregularities, and to monitor ovarian follicular function during induction of ovulation. In the female, Estradiol acts as a growth hormone for tissue of the reproductive organs. The development of secondary sexual characteristics in women is driven by Estradiol. Estradiol is involved also in men fertility. Estradiol regulates the bone maintenance. Post-menopause women experience an accelerated loss of bone mass due to a relative Estrogen deficiency. Estradiol affects the production of multiple proteins including lipoproteins, binding proteins, and proteins responsible for blood clotting. Estrogens have been found to have neuroprotective function. The Estradiol, for his activities, is involved in some types of cancer such as breast cancer and cancer of the uterine lining. In addition there are several benign gynaecologic conditions that are dependent on Estrogen such as endometriosis, leiomyomata uteri, and uterine bleeding.

2. INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of 17β-Estradiol in human serum or plasma.

3. PRINCIPLE OF THE ASSAY

Microtiter strip wells are precoated with anti-Estradiol antibodies (solid-phase). Estradiol in the sample competes with added horseradish peroxidase labelled Estradiol (enzyme-labelled antigen) for antibody binding. After incubation a bound/free separation is performed by solid-phase washing. The immune complex formed by enzyme-labelled antigen is visualized by adding Tetrathylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is inversely proportional to the amount of Estradiol in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Anti-Estradiol IgG Coated Wells:** 12 breakapart 8-well snap-off strips coated with anti-Estradiol IgG, in resealable aluminium foil.
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Estradiol-HRP conjugate:** 1 bottle containing 22 ml of horseradish peroxidase labelled Estradiol.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3, 3´, 5, 5´-tetramethylbenzidine (H2O2-TMB 0.26g/l) (avoid any skin contact).
- **Wash solution 10 x conc.:** 1 bottle containing 50 ml of a 10x concentrated solution of phosphate buffer 0.02 M (pH 7.4), NaCl 160 g/l, Tween-20 10 g/l
- **Estradiol Standards:** 6 bottles, 1 ml of Standard 0, 0,5 ml each of Standard 1 – 5
  - Standard 0: 0 pg/ml
  - Standard 1: 20 pg/ml
  - Standard 2: 120 pg/ml
  - Standard 3: 300 pg/ml
  - Standard 4: 600 pg/ml
  - Standard 5: 2000 pg/ml

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with anti-Estradiol IgG antibodies. Store at 2…8 °C. Open the bag only when it is at room temperature. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C, stability until expiry date. Do not remove the adhesive sheets on the unused strips.

6.2. Estradiol-HRP Conjugate

The conjugate is ready to use. Mix gently on a rotating mixer for 5 min. After first opening it is stable for another 6 months if stored at 2…8°C.

6.3. Estradiol Standards

The standard solutions are ready to use. 
After first use the standards are still stable for another 6 months if stored at 2...8°C.

6.4. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6.5. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

6.6. Wash Solution

Dilute the concentrated solution with distilled water to a final volume of 500 ml prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted solution is stable for 30 days at 2…8°C. In the concentrated solution it is possible to observe the presence of crystals, in this case mix at room temperature until complete dissolution of crystals. For greater accuracy dilute the whole bottle of concentrated wash solution to 500 ml and take care that all crystals are transferred by washing the bottle, then mix until crystals are completely dissolved.

7. SPECIMEN COLLECTION AND PREPARATION

The determination of Estradiol can be performed in plasma as well as in serum. If the assay is performed on the same day of sample collection, the specimen should be kept at 2...8°C; otherwise it should be aliquoted and stored deep-frozen (~-20 to -70°C). If samples are stored frozen, mix thawed samples gently for 5 min. before testing. Avoid repeated freezing and thawing.

7.1. Precaution

- The reagent contain Proclin 300® as preservative.
- Do not use heavily haemolysed samples.
- Maximum precision is required for dilution and dispensation of the reagents.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- This method allows the determination of Estradiol from 20 pg/ml to 2000 pg/ml.
- Treatment of the patient with cortisone, natural or synthetic steroids can impair Estradiol determination.
8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well (e.g. A1) for the substrate blank
2 wells (e.g. B1+C1) for standard 0
2 wells (e.g. D1+E1) for standard 1
2 wells (e.g. F1+G1) for standard 2
2 wells (e.g. H1+A2) for standard 3
2 wells (e.g. B2+C2) for standard 4
2 wells (e.g. D2+E2) for standard 5

It is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each standard and each patient sample.

Adjust the incubator to 37 °C.

1. Dispense 25 µl standards and samples into their respective wells. Add 200 µl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 2 hour at 37°C.
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100 µl TMB Substrate Solution into all wells.
6. Incubate for exactly 30 min at room temperature (22…28°C) in the dark.
7. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.
8. Measure the absorbance (E) of the specimen at 450 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and patient sample in the distribution and identification plan.

Where applicable calculate the mean absorbance values of all duplicates.

9. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Estradiol for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

10. RESULTS

10.1. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic). Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/ml.
10.2. Reference values
The serum Estradiol reference values:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Phase</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOMAN</td>
<td>Follicular phase</td>
<td>30 - 100 pg/ml</td>
</tr>
<tr>
<td></td>
<td>Ovulatory peak</td>
<td>130 - 350 pg/ml</td>
</tr>
<tr>
<td></td>
<td>Luteinic phase</td>
<td>50 - 180 pg/ml</td>
</tr>
<tr>
<td></td>
<td>Menopause</td>
<td>&lt; 60 pg/ml</td>
</tr>
<tr>
<td>MAN</td>
<td></td>
<td>&lt; 60 pg/ml</td>
</tr>
<tr>
<td>CHILDREN</td>
<td></td>
<td>&lt; 40 pg/ml</td>
</tr>
</tbody>
</table>

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

Intra Assay Variation
Within run variation was determined by replicate determination (10x) of two different control sera in one assay. The within assay variability is \( \leq 9\% \).

Inter Assay Variation
Between run variation was determined by replicate measurements of three different human sera in different lots. The between assay variability is \( \leq 10 \% \).

11.2. Cross Reactivity
The cross reaction of the antibody calculated at 50% according to Abraham:

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>100 %</td>
</tr>
<tr>
<td>Estrone</td>
<td>2.0 %</td>
</tr>
<tr>
<td>Estriol</td>
<td>0.39 %</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.02 %</td>
</tr>
<tr>
<td>Cortisol</td>
<td>( 7 \times 10^{-3} ) %</td>
</tr>
<tr>
<td>Progesterone</td>
<td>( 3 \times 10^{-4} ) %</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>( 1 \times 10^{-4} ) %</td>
</tr>
</tbody>
</table>

11.3. Sensitivity
The lowest detectable concentration of 17\( \beta \)-Estradiol calculated subtracting 2x S.D. to the media of ten replicates of standard 0 is 8.68 pg/ml.

11.4. Accuracy
The dilution test conducted with high concentration samples of 17\( \beta \)-Estradiol gave an average recovery value (± SD) of 95.69% ± 7.74% with reference to the original concentration.

The recovery of 120 – 240 - 480 – 960 pg/ml of Estradiol added to samples gave an average value (±SD) of 101.09 % ± 5.42 % with reference to the original concentrations.

11.5. Method comparison
The NovaTec ELISA was compared to another commercially available 17\( \beta \)-Estradiol assay. 16 serum samples were analysed in both test systems. The linear regression curve was calculated:

\[
(17 \text{ beta Estradiol NovaTec}) = 1.03 \times (17 \text{ beta Estradiol Reference}) - 12.96
\]

\[ r^2 = 0.996 \]

12. LIMITATIONS OF THE PROCEDURE
Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

| WARNING: | In the used concentration Proclin 300® has hardly any toxicological risk upon contact with skin and mucous membranes! |
| WARNING: | Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor! |

13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE

- Joshi, U.M. et al. (1979) Steroids 34(1), 35

15. ORDERING INFORMATION

Prod. No.: DNOV0003 Estradiol Determination (96 Determinations)
SCHEME OF THE ASSAY
17β - Estradiol

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

<table>
<thead>
<tr>
<th></th>
<th>Substrate blank</th>
<th>Standard 0</th>
<th>Standard 1</th>
<th>Standard 2</th>
<th>Standard 3</th>
<th>Standard 4</th>
<th>Standard 5</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
</tr>
<tr>
<td>Conjugate</td>
<td>-</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit

Incubate for 2 h at 37 °C

Wash each well three times with 300 µl diluted wash solution

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stop Solution</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Incubate for exactly 30 min at room temperature in the dark

Photometric measurement at 450 nm

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