Estrone

Enzyme immunoassay for the quantitative determination of Estrone in human serum or plasma

Only for in-vitro diagnostic use

Product Number: DNOV013 (96 Determinations)
1. INTRODUCTION

Estrone (also oestrone) is an estrogenic hormone secreted by the ovary. Estrone is one of the three estrogens, which also include estriol and estradiol. Estrone is the least prevalent of the three hormones, estradiol being prevalent almost always in a female body, estriol being prevalent primarily during pregnancy. Estrone sulfate acts as a pool of estrone which can be converted as needed to the more active estradiol.

In pre-menopausal adult women, more than 50% of estrone is secreted by the ovary. In pre-pubertal children, men and postmenopausal women, the major portion of estrone is derived from peripheral tissue conversion of androstenedione. During pregnancy, large amounts of estrone are synthesized in the placenta from dehydroepiandrosterone sulfate (DHEA-S) which originates from the maternal circulation and from the fetal adrenal gland. In pre-menopausal women, estrone levels generally parallel those of estradiol, rising gradually during the follicular phase, peaking just prior to ovulation, with a secondary and smaller increase during the luteal phase. After menopause, estrone levels do not decline as dramatically as estradiol levels, possibly due to increased conversion of androstenedione to estrone.

2. INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Estrone concentration in serum and plasma.

3. PRINCIPLE OF THE ASSAY

Microwell strip wells are precoated with anti-Estrone antibodies (solid-phase). Estrone in the sample competes with added horseradish peroxidase labelled Estrone (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 Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is inversely proportional to the amount of Estrone 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-Estrone Coated Wells**: 12 breakapart 8-well snap-off strips coated with anti-Estrone; in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Estrone-Biotin conjugate**: 1 bottle containing 0.2 ml of Biotin-labelled Estrone.
- **Enzyme Conjugate**: 1 bottle containing 0.2 ml peroxidase labelled Avidine.
- **Wash solution 50x conc.**: 1 bottle containing 20 ml (NaCl 9 g/l, Tween 20 1 g/l)
- **Incubation buffer**: 1 bottle containing 30 ml 50 mM phosphate buffer (pH 7.4; BSA 1 g/l)
- **TMB Substrate Solution**: 1 bottle containing 12 ml 3, 3’, 5, 5’-tetramethylbenzidine (H2O2-TMB 0.25g/l) (avoid any skin contact).
- **Estrone Standards**: 6 bottles, 1 ml each
  - Standard 0:       0 pg/ml
  - Standard 1:     15 pg/ml
  - Standard 2:     50 pg/ml
  - Standard 3:   200 pg/ml
  - Standard 4:   800 pg/ml
  - Standard 5  2000 pg/ml

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

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 20 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.
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-Estrone antibodies. Store at 2…8 °C. 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. Preparation of Conjugate
Prepare immediately before use.
Add 20 µl of Estrone-Biotin Conjugate and 20µl of Enzyme conjugate to 2.0 ml of Incubation Buffer.
Mix gently for 5 minutes with a vortex mixer.
Stable for 3 hours at room temperature.

6.3. Estrone Standards
Before use, mix for 2 min. with rotating mixer.
Once opened the standards are stable for six months at +4°C.

6.4. TMB Substrate Solution
The bottle contains 12 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. After first use the TMB substrate solution is still stable for another 6 months if stored at 2…8 °C.

6.5. Stop Solution
The bottle contains 12 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. After first use stable until expiry date.

6.6. Washing Solution
Dilute the concentrated wash solution 50x to 1000 ml with distilled or deionised water in a suitable storage container.
Store at room temperature. Stable until the expiry date printed on the label of the concentrated solution.

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma. If the assay is performed not perform on the day of sample collection the specimen should be stored at -20°C. If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

7.1. Precaution
- Do not use heavily haemolysed or lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- This method allows the determination of Estrone from 15 pg/ml to 2000 pg/ml.
- Treatment of the patient with cortisone, natural or synthetic steroids can impair Estrone 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 controls 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. 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. Please allocate at least:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 well (e.g. A1)</td>
<td>for the substrate blank</td>
</tr>
<tr>
<td>2 wells (e.g. B1+C1)</td>
<td>for standard 0</td>
</tr>
<tr>
<td>2 wells (e.g. D1+E1)</td>
<td>for standard 1</td>
</tr>
<tr>
<td>2 wells (e.g. F1+G1)</td>
<td>for standard 2</td>
</tr>
<tr>
<td>2 wells (e.g. H1+A2)</td>
<td>for standard 3</td>
</tr>
<tr>
<td>2 wells (e.g. B2+C2)</td>
<td>for standard 4</td>
</tr>
<tr>
<td>2 wells (e.g. D2+E2)</td>
<td>for standard 5</td>
</tr>
</tbody>
</table>
It is recommended to determine controls 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°C.

1. Dispense 50 µl standards and samples into their respective wells. Add 100 µl diluted 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 twice with 300µl 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 100µl TMB Substrate Solution into all wells.
6. Incubate for exactly 30 min at room temperature (22...28°C) in the dark.
7. Dispense 100µl 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. RESULTS

9.1. 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. (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.

9.2. Reference values
The following value for serum or plasma Estrone should be considered as a guideline:

<table>
<thead>
<tr>
<th>Category</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOMAN</td>
<td>25 - 350 pg/ml</td>
</tr>
<tr>
<td>PREGNANCY</td>
<td>100 - 1000 pg/ml</td>
</tr>
<tr>
<td>MEN</td>
<td>25 – 150 pg/ml</td>
</tr>
</tbody>
</table>

10. QUALITY CONTROL
Each laboratory should assay controls at normal, high and low levels range of Estrone 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.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision
Intra Assay Variation
Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 4.8 %.

Inter Assay Variation
Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 8.8 %.
11.2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham is:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>100%</td>
</tr>
<tr>
<td>16 epi-estriol</td>
<td>10.5%</td>
</tr>
<tr>
<td>15 αOH-estriol</td>
<td>7.0%</td>
</tr>
<tr>
<td>Estriol 3 Sulphate</td>
<td>2.0%</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.1%</td>
</tr>
<tr>
<td>17 epi-estriol</td>
<td>&lt; 1x10^-2%</td>
</tr>
<tr>
<td>Estriol 3 αGlucoronate</td>
<td>&lt; 1x10^-2%</td>
</tr>
<tr>
<td>Estriol 16 αGlucoronate</td>
<td>&lt; 1x10^-2%</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt; 1x10^-4%</td>
</tr>
</tbody>
</table>

11.3. Sensitivity

The lowest detectable concentration of Estrone that can be distinguished from the zero standard is 1 pg/ml at the 95% confidence limit.

11.4. Accuracy

The recovery of 20, 180, 160 pg/ml of Estrone added to sample gave an average value (±SE) of 99.6% ± 3.9% with reference to the original concentrations.

11.5 Correlation with RIA

The NovaTec Estrone ELISA was compared to another commercially available Estrone assay. Serum samples of 50 females were analysed according in both test systems. The linear regression curve was calculated

\[ y = 0.958x + 0.05 \]
\[ r = 0.96 \quad (r^2 = 0.92) \]

11.6. Hook Effect

The Estrone ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 40 ng/ml.

12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

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.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

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

15. ORDERING INFORMATION
Prod. No.: DNOV013 Estrone Determination (96 Determinations)
SCHEME OF THE ASSAY

Estrone

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>Blank</th>
<th>Stand. 0</th>
<th>Stand. 1</th>
<th>Stand. 2</th>
<th>Stand. 3</th>
<th>Stand. 4</th>
<th>Stand. 5</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stand. 1</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stand. 2</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stand. 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stand. 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stand. 5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50 µl</td>
</tr>
<tr>
<td>Conjugate</td>
<td>-</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>

Cover wells with foil supplied in the kit

**Incubate for 2 hour 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>
</tr>
</thead>
</table>

**Incubate for exactly 30 min at room temperature (22…28°C) in the dark**

<table>
<thead>
<tr>
<th>Stop Solution</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>
</table>

Photometric measurement at 450 nm

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