Estriol Saliva

Enzyme immunoassay for the quantitative determination of Estriol in saliva

Only for in-vitro diagnostic use

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Product Number: 40-521-475047 (96 Determinations)
CONTENTS

1. INTRODUCTION 3

2. INTENDED USE 3

3. PRINCIPLE OF THE ASSAY 3

4. MATERIALS 3

4.1. REAGENTS SUPPLIED 3

4.2. MATERIALS SUPPLIED 3

4.3. MATERIALS AND EQUIPMENT NEEDED 3

5. STABILITY AND STORAGE 3

6. REAGENT PREPARATION 4

6.1. COATED SNAP-OFF STRIPS 4

6.2. ESTRIOL-HRP CONJUGATE 4

6.3. ESTRIOL STANDARDS 4

6.4. TMB SUBSTRATE SOLUTION 4

6.5. STOP SOLUTION 4

7. SPECIMEN COLLECTION AND PREPARATION 4

7.1. PRECAUTION 4

8. ASSAY PROCEDURE 4

8.1. TEST PREPARATION 4

8.2. MEASUREMENT 5

9. RESULTS 5

9.1. STANDARD CURVE 5

9.2. CALCULATION OF RESULTS 5

9.3. REFERENCE VALUES 6

10. SPECIFIC PERFORMANCE CHARACTERISTICS 6

10.1. PRECISION 6

10.2. CROSS REACTIVITY 6

10.3. ANALYTIC SENSITIVITY 6

10.4. ACCURACY 6

10.5. METHOD COMPARISON 6

11. LIMITATIONS OF THE PROCEDURE 6

12. PRECAUTIONS AND WARNINGS 7

12.1. DISPOSAL CONSIDERATIONS 7

13. LITERATURE 7

14. ORDERING INFORMATION 7
1. INTRODUCTION

Estriol is a female sex steroid hormone and one of the three major naturally occurring estrogens, the others being estradiol and estrone. Estriol is produced almost exclusively during pregnancy and is the major estrogen produced in the normal human fetus. During pregnancy the production of Estriol depends on an intact maternal-placental-fetal unit. Fetal-placental production of Estriol leads to a progressive rise in maternal circulating levels reaching a late-gestational peak several orders of magnitude greater than non-pregnant levels. In non-pregnant circumstances, Estriol is derived almost exclusively from 17β-Estradiol. Estriol exists in biological matrix in free (ca. 9 %) and conjugated form (91 %). Conjugation with glucuronic or sulphuric acid of estriol occurs in the liver, which aids in steroid solubility, therefore allowing rapid elimination via the kidney.

2. INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Estriol in saliva.

3. PRINCIPLE OF THE ASSAY

Microtiter strip wells are precoated with anti-Estriol antibodies (solid-phase). Estriol in the sample competes with added horseradish peroxidase labelled Estriol (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 Estriol 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-Estriol IgG Coated Wells: 12 breakapart 8-well snap-off strips coated with anti-Estriol IgG, in resealable aluminium foil.
- Stop Solution: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- Estriol-HRP conjugate conc.: 1 bottle containing 0.4 ml of horseradish peroxidase labelled Estriol.
- TMB Substrate Solution: 1 bottle containing 12 ml 3, 3’, 5, 5’-tetramethylbenzidine (H2O2-TMB 0.25g/l) (avoid any skin contact).
- Incubation buffer: 1 bottle containing 30 ml phosphate buffer.
- Estriol Standards: 5 bottles, 1 ml each
  - Standard 0: 0 pg/ml
  - Standard 1: 0.1 pg/ml
  - Standard 2: 1.0 pg/ml
  - Standard 3: 4.0 pg/ml
  - Standard 4: 12.0 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 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Glass 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 (20…25°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-Estriol 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. Estriol-HRP Conjugate
Prepare immediately before use. Add 10 µl stock solution to 2 ml Incubation buffer. Mix gently. Stable for 3 hours at room temperature (+18…+25°C).

6.3. Estriol Standards
Before use, mix for 5 min. with a rotating mixer.

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.

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.

7. SPECIMEN COLLECTION AND PREPARATION

The determination of Estriol can be performed in saliva. It is recommended to collect saliva samples with a centrifuge glass tube and a plastic straw:
let the saliva flow down through the straw into the centrifuge glass tube; centrifuge at 3000 rpm for 5 min.
SALIVETTE collector device can be used in Cortisol Saliva assay. Other equipment of sample collection commercially available has not been tested.
At 2…8 °C the samples can be stored for one week. For longer periods samples should be stored at -20 °C. Repeated freeze-thawing should be avoided. Thawed samples should be inverted several times prior to use.

7.1. Precaution
- The reagents contain Proclin 300® (0.01 %) as preservative.
- Maximum precision is required for dilution and dispensation of the reagents.
- This method allows the determination of Estriol from 0.1 pg/ml to 12.0 pg/ml.
- For concentration of Estriol over 12 pg/ml dilute the sample (1+3) with Incubation buffer. Consider the dilution factor when calculating the results.
- Treatment of the patient with natural or synthetic steroids can impair Estriol 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:

<table>
<thead>
<tr>
<th>Number of Wells</th>
<th>Well(s)</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(e.g. A1)</td>
<td>for the substrate blank</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. B1+C1)</td>
<td>for standard 0</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. D1+E1)</td>
<td>for standard 1</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. F1+G1)</td>
<td>for standard 2</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. H1+A2)</td>
<td>for standard 3</td>
</tr>
<tr>
<td>2</td>
<td>(e.g. B2+C2)</td>
<td>for standard 4</td>
</tr>
</tbody>
</table>
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 20 µl standards and samples into their respective wells. Add 200 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 1 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 300 µl distilled water. 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 15 min at +20…+25°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. RESULTS

9.1. Standard curve
- A log-logit curve fitting model for calculating the Estriol concentrations of the samples is recommended.
- Calculate the percentage of maximal binding (%B/B0 value) of each standard by dividing the absorbance of the standard (S1 - S4) by the B0 absorbance (S0) and multiply by 100. Transform the ratio into the logit function, where
  \[ \text{logit} = \ln \left(\frac{\%B/B_0}{100 - \%B/B_0}\right) \]
- Example: B0 absorbance = 1.800 = B0
  - 4 pg/ml standard absorbance = 1.000
  - \(\%B/B_0 = 1.000/1.800 \times 100 = 55.5\)
  - logit = ln (55.5/(100-55.5)) = 0.221
- Repeat for all standards.
- Graph the standard curve by plotting the logit for each standard concentration on the ordinate (y-axis) against the log of the standard concentrations on the abscissa (x-axis).

9.2. Calculation of Results
- Determine the %B/B0 and logit values for each sample.
- Using the standard curve, the concentration of each sample can be determined by comparing the logit value of each sample with the corresponding concentration of Estriol standard.
- If the samples were diluted, the concentrations determined from the standard curve must be multiplied by the dilution factor.

All suitable computer programs available can be used for automated result reading and calculation. The following mathematical functions can be used: Logit-log calculation.
9.3. Reference values
Each laboratory must establish its own normal ranges based on patient population.
The following values for Estriol should be considered as a guideline:

<table>
<thead>
<tr>
<th>Pregnancy weeks</th>
<th>&quot;unconjugated Estriol&quot; in saliva (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td>24</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>26</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>28</td>
<td>1.5 ± 0.8</td>
</tr>
<tr>
<td>30</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>32</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>34</td>
<td>3.2 ± 1.3</td>
</tr>
<tr>
<td>36</td>
<td>4.1 ± 1.6</td>
</tr>
<tr>
<td>37</td>
<td>4.5 ± 1.7</td>
</tr>
<tr>
<td>38</td>
<td>5.0 ± 2.0</td>
</tr>
<tr>
<td>39</td>
<td>5.3 ± 2.0</td>
</tr>
<tr>
<td>40</td>
<td>5.7 ± 2.0</td>
</tr>
</tbody>
</table>

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision
The inter- and intra-run precision had a coefficient of variation of 3.6% and .6.1% respectively.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estriol</td>
<td>100.0 %</td>
</tr>
<tr>
<td>16-epi-estriol</td>
<td>10.5 %</td>
</tr>
<tr>
<td>15 α-OH-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 α-glucuronate</td>
<td>&lt;1x10⁻³ %</td>
</tr>
<tr>
<td>Estriol-16 α-glucuronate</td>
<td>&lt;1x10⁻⁴ %</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;1x10⁻⁸ %</td>
</tr>
</tbody>
</table>

10.3. Analytic Sensitivity
The sensitivity of this method is 2 pg/ml, when the value of (B/B₀) % is approx. 90% (calculated as two times the S.D. from B₀).

10.4. Accuracy
The recovery of 0.1 – 1.0 – 4.0 – 12.0 pg/ml of Estriol added to sample of saliva gave an average value (±SE) of 104 % ± 6.0% with reference to the original concentrations.

10.5. Method comparison
Correlation with RIA performed on the same samples:

\[ y = 0.90 + 1.12 \times \]

\[ r = 0.996 \]

\[ n = 25 \]

\[ y = < 0.001 \]

11. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.
12. 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! |

12.1. 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.

13. LITERATURE


14. ORDERING INFORMATION

Prod. No.: 40-521-475047 Estriol Saliva Determination (96 Determinations)
SCHEME OF THE ASSAY
Estriol Saliva

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>Substrate blank</th>
<th>Standard 0</th>
<th>Standard 1</th>
<th>Standard 2</th>
<th>Standard 3</th>
<th>Standard 4</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation buffer</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
<td>-</td>
</tr>
<tr>
<td>Standard 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 µl</td>
</tr>
<tr>
<td>Diluted Conjugate</td>
<td>-</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 1 h at 37 °C**
Wash each well twice with 300 µl distilled water

<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>
</tr>
</thead>
</table>

**Incubate for exactly 15 min at +20...+25 °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>
</tr>
</thead>
</table>

Photometric measurement at 450 nm