Estriol Saliva ELISA

Enzyme immunoassay for the quantitative determination of Estriol in human saliva.

REF RE52291

Σ 96

2-8°C

EU: IVD
1 INTENDED USE
Enzyme immunoassay for the quantitative determination of Estriol in human saliva.

2 CLINICAL SIGNIFICANCE
Estriol (also Oestriol) is one of the three main estrogens produced by the human body. It is only produced in significant amounts during pregnancy as it is made by the 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 lategestational peak several orders of magnitude greater than non-pregnant levels. In the maternal circulation, estriol undergoes a rapid conjugation in the liver followed by urinary excretion with a half-life of about 20 minutes. Since normal estriol production depends on an intact maternal-placental-fetal circulation and functional fetal metabolism, maternal estriol levels have been used to monitor fetal status during pregnancy, particularly during the third trimester. DHEA-S is produced by the adrenal cortex of the fetus, this is converted to estriol by the placenta. If levels are abnormally low in a pregnant woman, this may indicate a problem with the development in the child. Levels of estriol in non-pregnant women do not change much after menopause, and levels are not significantly different from levels in men.

3 PRINCIPLE
Estriol (antigen) in the sample competes with horseradish peroxidase estriol (enzyme-labelled antigen) for binding onto the limited number of antiestriol (antibody) sites on the microplate (solid phase). After incubation, the bound/free separation is performed by a simple solid-phase washing. Then, the enzyme HRP in the bound-fraction reacts with the Substrate (H2O2) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H2SO4) is added. The colour intensity is inversely proportional to the Estriol concentration in the sample. Estriol concentration in the sample is calculated through a calibration curve.

4 REAGENTS, MATERIALS AND INSTRUMENTATION

4.1 REAGENTS AND MATERIALS SUPPLIED IN THE KIT
1. Coated Microplate 1x (breakable)
   MTP Anti-Estriol IgG adsorbed on microplate
2. Conjugate (1 vial) 1 mL
   ENZCONJ Conc Estriol-HRP conjugate
3. Estriol Standards 6x (1 vial = 1 mL)
   CAL 0 - 5
4. TMB-substrate (1 vial) 15 mL
   TMB SUBS H2O2-TMB 0.26 g/L (avoid any skin contact!)
5. Stop solution (1 vial) 15 mL
   STOP 0.15 mol/L H2SO4 (avoid any skin contact!)
6. Wash solution Conc. 50X (1 bottle) 20 mL
   WASH CONC NaCl 45 g/L; Tween-20 55 g/L
7. Incubation Buffer (1 bottle) 30 mL
   INCBuf Phosphate buffer pH 7.5; BSA 1g/L
8. Controls (2 vials = 1mL each)
   Control L
   Control M

4.2 REAGENTS NECESSARY NOT SUPPLIED
Distilled water
4.3 AUXILIARY MATERIALS AND INSTRUMENTATION

Automatic dispenser.
Microplates reader (450 nm, 620-630nm)
Saliva Collection Device

Note

Store all reagents at 2-8 °C in the dark.
Open the bag of Coated Microplate only when it is at room temperature and close immediately after use.
The microplate, once opened, it stable until the expire date of kit. Do not remove the adhesive sheets on the unused strips.

5 WARNINGS

• This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
• Use appropriate personal protective equipment while working with the reagents provided.
• Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws..
• Some reagents contain small amounts of Proclin 300® as preservative. Avoid the contact with skin or mucosa.
• The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
• The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
• Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.
• This method allows the determination of Estriol from 2.5 pg/mL to 4000 pg/mL.
• The clinical significance of Estriol determination can be invalidated if the patient was treated with natural or synthetic steroids.

6 PRECAUTIONS

• Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
• All reagents should be stored refrigerated at 2-8 °C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
• Allow all kit components and specimens to reach room temperature (22-28 °C) and mix well prior to use.
• Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
• If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested.
• The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
• 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 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
• Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
• Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
• Maximum precision is required for reconstitution and dispensation of the reagents.
• Samples microbiologically contaminated, highly lipemic or haemolysed specimens should not be used in the assay.
• Plate readers measure vertically. Do not touch the bottom of the wells.
## 7 PROCEDURE

### 7.1 Preparation of the Standard (CAL 0…CAL 5)

Before use, mix for 5 min. with rotating mixer.
The standard has the following concentration of Estriol:

<table>
<thead>
<tr>
<th>CAL</th>
<th>pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
</tr>
<tr>
<td>5</td>
<td>4000</td>
</tr>
</tbody>
</table>

Once open, the standards are stable at 2-8 °C for 6 months.
For SI UNITS: pg/mL x 3.5 = pmol/mL

### 7.2 PREPARATION OF THE WASH SOLUTION

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2-8 °C.

### 7.3 PREPARATION OF DILUTED CONJUGATE

Prepare immediately before use.
Add 10 μL Conjugate to 1.0 mL of Incubation Buffer. Mix gently. Stable 3 hours at 22-28 °C.

### 7.4 PREPARATION OF THE SAMPLE

The determination of Estriol with this kit should be performed in saliva samples. Suitable sampling device should be used.

#### 7.4.1 METHOD AND LIMITATIONS

Collect saliva samples at the times indicated. In order to have high reproducibility and accuracy, it is advisable to collect at least 3 samples in a period of not less than 2 hours and pooling the samples before testing.

If no specific instructions have been given oral fluid (saliva) samples may be collected at any time; for saliva collection, the following should be noted:

a) If saliva collection is carried out in the morning ensure that this is carried out prior to brushing teeth

b) During the day allow 1 hour after any food or drink before collecting saliva samples

c) It is very important that a good clear sample is received – i.e. no contamination with food, lipstick, blood (bleeding gums) or other extraneous materials.

#### 7.4.2 SALIVA PROCESSING INSTRUCTIONS

Let the saliva flow down through the straw into the centrifuge glass tube

1. Centrifuge the sample for 15 minutes at 3000 rpm
2. Store at – 20 °C for at least 1 hour
3. Defrost samples
4. Centrifuge again for 15 minutes at 3000 rpm
5. The saliva sample is now ready to be tested.
6. Store the sample at 2-8 °C for one week or at – 20 °C for longer time.
7.5 PROCEDURE

Allow all reagents to reach room temperature (22-28°C). At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.

Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

As it is necessary to perform the determination in duplicate, prepare two wells for each point of the standard curve (CAL 0 - CAL 5), two wells for each control and for each sample, one for Blank.

Pipette:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standard</th>
<th>Sample / Controls</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards CAL 0 - CAL 5</td>
<td>50 µL</td>
<td>50 µL</td>
<td></td>
</tr>
<tr>
<td>Sample Control L+M</td>
<td></td>
<td>50 µL</td>
<td></td>
</tr>
<tr>
<td>Diluted Conjugate</td>
<td>100 µL</td>
<td>100 µL</td>
<td></td>
</tr>
</tbody>
</table>

Incubate 60 minutes at 22-28 °C.

Remove the contents from each well. Wash the wells 3 times with 300 µL of diluted wash solution.

Important note: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

Automatic washer: if you use automated equipment, wash the wells at least 5 times.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Incubate at room temperature 22-28 °C for 15 minutes in the dark.

<table>
<thead>
<tr>
<th>Stop solution</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Shake the microplate gently.

Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.

8 QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Estriol for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be 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.

9 RESULTS

9.1 MEAN ABSORBANCE

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample.

9.2 STANDARD CURVE

Plot the mean value of absorbance of the standards (Em) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

9.3 CALCULATION OF RESULTS

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.
10 REFERENCE VALUE
As the Estriol Saliva values follow a circadian pattern we suggest to collect the samples at the same time (8 A.M.):
The following values should be used as preliminary guide until each laboratory has your own reference range.

<table>
<thead>
<tr>
<th>Women, premenopausal</th>
<th>Time</th>
<th>N</th>
<th>Range ± 2SD [pg/mL]</th>
<th>Absolute Range [pg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8:00</td>
<td>21</td>
<td>0 – 21.0</td>
<td>0 – 32.0</td>
</tr>
<tr>
<td></td>
<td>17:00</td>
<td>21</td>
<td>0 – 6.8</td>
<td>0 – 8.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pregnancy weeks</th>
<th>Estriol in Saliva (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>(700 ± 500)</td>
</tr>
<tr>
<td>24</td>
<td>(900 ± 600)</td>
</tr>
<tr>
<td>26</td>
<td>(1200 ± 700)</td>
</tr>
<tr>
<td>28</td>
<td>(1500 ± 800)</td>
</tr>
<tr>
<td>30</td>
<td>(1800 ± 800)</td>
</tr>
<tr>
<td>32</td>
<td>(2200 ± 1100)</td>
</tr>
<tr>
<td>34</td>
<td>(3200 ± 1300)</td>
</tr>
<tr>
<td>36</td>
<td>(4100 ± 1600)</td>
</tr>
<tr>
<td>37</td>
<td>(4500 ± 1700)</td>
</tr>
<tr>
<td>38</td>
<td>(5000 ± 2000)</td>
</tr>
<tr>
<td>39</td>
<td>(5300 ± 2000)</td>
</tr>
<tr>
<td>40</td>
<td>(5700 ± 2000)</td>
</tr>
</tbody>
</table>

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

11 PERFORMANCE AND CHARACTERISTICS

11.1 PRECISION

11.1.1 INTRA ASSAY VARIATION
Within run variation was determined by replicate measurements (16x) of two different saliva control in one assay. The within assay variability is 9.7%.

11.1.2 INTER ASSAY VARIATION
Between run variation was determined by replicate measurements (10x) of two different saliva control with different lots of kit. The between assay variability is ≤13.7%.

11.2 ACCURACY
The recovery of 50, 300, 2000 ng/mL of Estriol added to “saliva-free” sample gave an average value (±SD) of 100.6% ± 14.6% with reference to the original concentrations.

11.3 SENSITIVITY
The lowest detectable concentration of Estriol that can be distinguished from the zero standard is 1.0 pg/mL at the 95% confidence limit.
11.4 SPECIFICITY
The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Crossreactivity</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>15α-OH-estriol</td>
<td>7.0%</td>
</tr>
<tr>
<td>Estriol-3-sulfat</td>
<td>2.0%</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.1%</td>
</tr>
<tr>
<td>17-epi-estriol</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Estriol-3α-glucuronat</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Estriol-16α-glucuronate</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Prednisone</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Estron</td>
<td>&lt;0.0001%</td>
</tr>
</tbody>
</table>

11.5 CORRELATION
The Estriol saliva ELISA kit was compared to another commercially available Estriol saliva assay. 30 saliva samples were analysed according in both test systems.

The linear regression curve was calculated:
\[ y = 1.03x + 0.68 \]
\[ r^2 = 0.988 \]
\[ y = \text{Estriol Saliva ELISA (IBL)} \]
\[ x = \text{Estriol Saliva ELISA (Salimetrics)} \]

12 WASTE MANAGEMENT
Reagents must be disposed off in accordance with local regulations.

13 BIBLIOGRAPHY
<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός-Παραγωγή:</td>
</tr>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
<tr>
<td></td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
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<td>LYO</td>
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<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
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</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύμβολα των συστατικών του κιτ συμβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

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