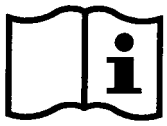
Product information



Useŕs Manual

Estriol free in Saliva ELISA

REF **IB79313**

96 Wells

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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INTENDED USE
Competitive immunoenzymatic colorimetric method for the determination of Estriol in saliva.
For research use only, not for use in diagnostic procedures.

1. Introduction

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 late-gestational 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.

2. PRINCIPLE

The Estriol (antigen) in the sample competes with the antigenic Estriol conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti Estriol coated 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 (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution 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.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with a anti-Estriol antibody.
2. ***Standard (Standard 0-5),*** 6 vials, 1 ml, ready to use;
   Concentrations: 0 – 2.5 – 15 – 100 – 600 – 4000 pg/ml.
   Conversion: pg/ml x 3.5 = pmol/L
3. ***Control*** L & M, 2 vials, 1.0 ml, ready to use;
   For control values and ranges please refer to QC-Datasheet.
4. ***Enzyme Conjugate concentrate***, 1 vial,1 ml,
   Estriol conjugated to horseradish peroxidase,
   see „Preparation of Reagents“.
5. ***Incubation Buffer***, 1 vial, 30 ml, ready to use.
   Phosphate buffer pH 7.5, BSA 1g/l
6. ***Substrate Solution***, 1 vial, 15 ml, ready to use;
   Tetramethylbenzidine (TMB).
7. ***Stop Solution***, 1 vial, 15 ml, ready to use;
   contains 0.3 N/l acidic solution.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
8. ***Wash Solution***, 1 vial, 20 ml (50X concentrated);
   NaCl 45 g/L; Tween-20 55g/L
   see „Preparation of Reagents“.

3.1. Reagents necessary not supplied

Distilled water

3.2. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Note

Store all reagents at 2÷ 8°C in the dark.

Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use.

Once opened, the microplate is stable until the expiry date of the kit. Do not remove the adhesive sheets on the unused strips

4. WARNINGS

- This kit is intended for research us only.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300^{R} 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/H_2O_2 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 significance of Estriol determination can be invalidated if the unknown was treated with natural or synthetic steroids.

5. 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 samples to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the 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.
- 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 lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

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6. PROCEDURE

6.1. Preparation of the Standard (S_0…S_5)

Before using, mix for 5 min with a rotating mixer.
The standards are ready to use and have the following concentration of Estriol:

	S_0	S_1	S_2	S_3	S_4	S_5
pg/mL	0	2,5	15	100	600	4000

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

6.2. 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.

6.3. Preparation of Diluted Conjugate

Prepare immediately before use.
Add 10 µL Conjugate (reagent 4) to 1.0 mL of Incubation Buffer (reagent 3). Mix gently. Stable 3 hours at room temperature (22÷28°C).

6.4. Preparation of the Sample

Samples containing sodium azide should not be used in the assay. The saliva samples should be completely colorless. Even the slightest red color shows blood contamination resulting in falsely elevated concentration values. In case of visible blood contamination the individual should discard the sample, rinse the sampling device with water, wait for 10 minutes and take a new sample.

Sample Collection

For the correct collection of saliva we are recommending to only use appropriate devices made from ultra-pure polypropylene. Do not use any PE devices or cotton based Salivettes for sampling. False readings will result. Glass tubes can be used as well, but in this case special attention is necessary for excluding any interference caused by the stopper. Please contact IBL-America for more details.
As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting. If fasting should be a problem at least any food of animal origin (meat or dairy products) should be avoided prior to finalizing the collection. In the morning breakfast should be done only after finalizing the collection procedure. During the day the collection period should be timed just before an anticipated meal. Drinking of coffee is not allowed during the last 3 hours before taking the samples. As the steroid hormone secretion in saliva as well in serum shows an obvious dynamic secretion pattern throughout the day it is important to always collect 5 samples during a 2 hour period; this means every 30 minutes one sample. If possible the volume of each single sample should be a minimum of 0.5 ml (better 1 ml). Saliva flow may be stimulated by drinking water. This is allowed and even recommended before and during the collection period. Drinking of water is not allowed during the last 5 minutes before taking the single samples.

Sample Storage and Preparation

Saliva samples in general are stable at ambient temperature for up to seven days. Therefore mailing of such samples by ordinary mail without cooling will not create any problem. Storage at 4°C can be done for a period of up to one month. Whenever possible samples should preferably be kept at a temperature of -20°C. Even repeated thawing and freezing is not a problem. Each sample has to be frozen, thawed, and centrifuged at least once anyhow in order to precipitate the mucins by centrifugation. Upon arrival of the samples at the lab the samples have to kept frozen at least overnight. Next morning the samples are thawed and mixed carefully. The samples have to be centrifuged for 5 to 10 minutes. The clear colorless supernatant is easy to pipette. If the sample should show even a slighty red color it should be discarded. Otherwise the value most probably will be falsely elevated. Due to the episodic variations of the steroid secretion we highly recommend the strategy of multiple sampling. If such a set of multiple samples has to be tested the staff of lab (after at least one freezing, thawing, and centrifugation cycle) should mix aliquots of the 5 single samples and perform the determination using the mixture.

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C).**
- 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 in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample/ Controls	Blank
Standard S_0-S_5	**50 µL**		
Sample/ Control L-M		**50 µL**	
Diluted Conjugate	**100 µL**	**100 µL**	
Incubate 1 h at room temperature (22÷28°C). Remove the contents from each well; wash the wells 3 times with 300 µL of diluted wash solution.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22÷28°C) for 15 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

7. 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 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 Calibration 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.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the Calibration curve (C_0-C_5) and of each sample.

8.2. Calibration curve

Plot the mean value of absorbance (Em) of the Standards (S_0-S_5) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the Calibration curve to obtain the corresponding values of the concentrations expressed in pg/mL.

9. PERFORMANCE AND CHARACTERISTICS

9.1. Precision

9.1.1. Intra Assay Variation

Within run variation was determined by replicate (16x) the measurement of two different saliva control in one assay. The within assay variability is ≤ 9.7%.

9.1.2. Inter Assay Variation

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

9.2. Accuracy

The recovery of 50 – 300 – 2000 pg/mL of Estriol added to "saliva-free" sample gave an average value (±SD) of 100.6% ± 14.6% with reference to the original concentrations.

9.3. Sensitivity

The lowest detectable concentration of Estriol that can be distinguished from the Standard 0 is 1.0 pg/mL at the 95 % confidence limit.

9.4. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Estriol saliva	100 %
16 epi-estriol	10.5 %
15 α-OH-estriol	7.0 %
Estriol 3-Sulfate	2.0 %
Estradiol	0.1 %
17 epi-estriol	< 1x10-2 %
Estriol 3α-Glucoronide	< 1x10-2 %
Estriol 16α-Glucoronide	< 1x10-2 %
Prednisone	$< 1x10^{-2}$ %
Estrone	$< 1x10^{-4}$ %

9.5. Correlation

Demeditec Estriol saliva ELISA kit was compared to another commercially available Estriol saliva assay. 30 saliva samples were analysed according to both test systems. The linear regression curve was calculated:

$y = 1.03x + 0.68$
$r^2 = 0.988$
y = Estriol saliva IBL-America Elisa kit
x = Estriol saliva Salimetrics Elisa kit

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

Manufactured for :

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