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Instructions for use

Estriol Saliva ELISA Free

REF SA E-6600

96

+2 +8 °C

IVD

CE

Introduction

Intended Use

The **Salivary Free Estriol ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of free estriol in saliva.

Summary and Explanation

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.

PRINCIPLE of the test

The Salivary Free Estriol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with an anti-Estriol IgG antibody. Endogenous unconjugated ("free") Estriol of a patient sample competes with an Estriol-horseradish peroxidase conjugate for binding to the coated antibody.
After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estriol in the sample.
After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of free Estriol in the patient sample.

Precautions

1. This kit is for in vitro diagnostic use only. For professional use only.
2. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
3. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Avoid contact with *Stop Solution* containing 0.15 M H_2SO_4. It may cause skin irritation and burns.
5. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
6. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
7. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
8. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
9. Do not use reagents beyond expiry date as shown on the kit labels.
10. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
11. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
12. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
13. Safety Data Sheets for this product are available upon request directly from the manufacturer.
    The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

REAGENT, MATERIAL AND INSTRUMENTATION

Reagent and material supplied in the kit

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
STANDARD A	**SA E-6601**	Standard 0	0 pg/ml	1 ml
STANDARD B	**SA E-6602**	Standard 1	2.5 pg/ml	1 ml
STANDARD C	**SA E-6603**	Standard 2	15 pg/ml	1 ml
STANDARD D	**SA E-6604**	Standard 3	100 pg/ml	1 ml
STANDARD E	**SA E-6605**	Standard 4	600 pg/ml	1 ml
STANDARD F	**SA E-6606**	Standard 5	4000 pg/ml	1 ml

Conversion: pg/mL x 3.5 = pmol/L

CONTROL 1 **+** CONTROL 2 **SA E-6651** + **SA E-6652 Control L and M**

2 vials, 1.0 mL each, ready to use; For control values and ranges please refer to vial label or QC-Datasheet. Contain a non-mercury preservative.

INC-BUFF **SA E-6613 Incubation Buffer**

1 vial, 30 mL, ready to use. Phosphate buffer pH 7.4, BSA 1g/L

CONJUGATE-CONC **SA E-6640 Enzyme Conjugate concentrate**

1 vial,1 mL, Estriol conjugated to horseradish peroxidase, see „Preparation of Reagents".

96 **SA E-6631 Coated Microplate**

Wells coated with a anti-Estriol IgG antibody.

WASH-CONC 50x **SA E-0030 Conc. Wash Solution 50X**

1 vial, 20 mL (50X concentrated); contains Phosphate buffer 50 mM pH 7.4; Tween20 1gr/l,
see „Preparation of Reagents".

SUBSTRATE **SA E-6655 TMB-Substrate**

1 vial, 15 mL, ready to use; Tetramethylbenzidine (TMB).

STOP-SOLN **SA E-6680 Stop Solution**

1 vial, 15 mL; Sulphuric acid 0.15 mol/L (avoid any skin contact)

Equipment and material required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Saliva Collection Device
- Semi logarithmic graph paper or software for data reduction

Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Standards

Before use, mix the standards for 5 minutes with a rotating shaker.

Wash Solution

Add deionized water to the 50X concentrated Wash Solution.
Dilute concentrated *Wash Solution* (20 mL) with distilled or deionized water to a final volume of 1000 mL.
Store at room temperature until expiration date printed on label of the concentrate vial.

Enzyme Conjugate

Add 10 µl Enzyme Conjugate concentrate to 1.0 mL of Incubation Buffer. Mix gently.
Stability of the prepared Enzyme-Conjugate : Stable for 3 hours at 22°C - 28°C
Prepare immediately before use.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN Collection and Preparation

Eating, drinking, chewing gums or brushing teeth should be avoided for 30 minutes before sampling. Otherwise, it is recommended to rinse mouth thoroughly with cold water 5 minutes prior to sampling.
Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).
If there is visible blood contamination the patient specimen, it should be discarded, rinse the sampling device with water, wait for 10 minutes and take a new sample.
Note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Saliva samples should be collected only using special saliva sampling devices (vial and straw),
: e.g. SALI SET 100 REF SA D-6100 available from LDN. Do not use Salivette (cotton swab) for sampling.
Due to the cyclic secretion pattern of steroid hormones it is important to care for a proper timing of the sampling.
In order to avoid arbitrary results we recommend that 5 samples always be taken within a period of 2 – 3 hours (*multiple sampling*) preferably before a meal.
As food might contain significant amounts of steroid hormones samples preferably should be taken while fasting.
If fasting should be a problem the collection period should be timed just before lunch or before dinner.
NOTE: The clinical significance of the Estriol determination can be invalid if the patient was treated with natural or synthetic steroids.

Specimen Storage and Preparation

The saliva samples may be stored at 2 °C to 8 °C up to one week, and should be frozen at –20 °C for longer periods. Repeated thawing and freezing should be avoided.

Each sample has to be frozen, thawed, and centrifuged at least once in order to separate the mucins by centrifugation.
Upon arrival of the samples in the lab the samples have to stay in the deep freeze at least overnight. Next morning the frozen samples are warmed up to room temperature and mixed carefully.
Then the samples have to be centrifuged for 5 to 10 minutes (at 2000 - 3000 x g).
Now the clear colorless supernatant is easy to pipette.

If a set of multiple samples is to be tested, the lab (after at least one freezing, thawing, and centrifugation cycle) has to mix the 5 single samples in a separate sampling device and perform the testing from this mixture.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Incubation Buffer* (1+3) and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1+3: 10 µL saliva + 30 µL *Incubation Buffer* (mix thoroughly)

Assay procedure

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL** of diluted ***Enzyme Conjugate*** into each well. (See "Preparation of Reagents".)
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature (22°C – 28°C).
5. Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of ***Substrate Solution*** to all well.
7. Incubate for **15 minutes** at room temperature (22°C – 28°C) in the dark.
8. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to all wells. Thoroughly mix for 10 seconds.
9. Read the absorbance (OD) of each well at **450±10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

Expected Normal values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

As the salivary estriol 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.

Population		N	range ± 2 SD [pg/mL]	absolute range [pg/mL]
Women, premenopausal,	8 AM	21	0 – 21.0	0 – 32.0
	5 PM	21	0 – 6.8	0 – 8.9

Pregnancy week	Free Estriol (pg/mL) in saliva
22	(700 ± 500)
24	(900 ± 600)
26	(1200 ± 700)
28	(1500 ± 800)
30	(1800 ± 800)
32	(2200 ± 1100)
34	(3200 ± 1300)
36	(4100 ± 1600)
37	(4500 ± 1700)
38	(5000 ± 2000)
39	(5300 ± 2000)
40	(5700 ± 2000)

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

Performance Characteristics

Assay Dynamic Range

The range of the assay is between 2.5 – 4000 pg/mL.

Specificity of Antibodies (Cross Reactivity)

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

Substance	Cross Reactivity
estriol	100.0%
16-epi-estriol	10.5%
15α-OH-estriol	7.0%
estriol -3-sulphate	2.0%
estradiol	0.1%
17-epi-estriol	< 0.01%
estriol-3α-glucuronate	< 0.01%
estriol-16α-glucuronate	< 0.01%
estrone	< 0.0001%

Analytical Sensitivity

The lowest detectable concentration of Estriol that can be distinguished from the zero standard is 1.1 pg/mL at the 95 % confidence limit

Precision

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

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

Limitations of Use

Any improper handling of samples or modification of this test might influence the results.

Drug Interferences

The clinical significance of Estriol determination can be invalidated if the patient was treated with natural or synthetic steroids.

High-Dose-Hook Effect

No hook effect was observed in this test.

Legal Aspects

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacturer.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

REFERENCES

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!