Estrone ELISA (Saliva)

For the quantitative determination of estrone in saliva.

For Research use Only. Not For Use In Diagnostic Procedures.

Please read carefully due to Critical Changes, e.g., Assay characteristics, dynamic range and cross reactivity.

Catalog Number: 20-ESRHU-E01-SLV
Size: 96 wells
Version: 2.1 2017/06-ia – ALPCO June 19, 2017

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1 INTRODUCTION

1.1 Intended Use

The **Salivary Estrone ELISA** is an enzyme immunoassay for the quantitative measurement of estrone in saliva.

2 PRINCIPLE OF THE TEST

The Salivary Estrone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with streptavidin and biotinylated estrone molecules. Endogenous estrone in a sample competes with this immobilized estrone for binding sites of an HRP-conjugated sheep antibody directed towards an antigenic site of estrone. After incubation, the unbound antibody conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of estrone in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of estrone in the sample.

3 Warnings and Precautions

1. This kit is for research use only. For professional use only.
2. 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.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
13. Handling should be done in accordance with the procedures defined by appropriate national biohazard safety guidelines or regulations.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. 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 be slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.

18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard safety guidelines or regulations.
21. For information on hazardous substances included in the kit please refer to the Safety Data Sheet.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiter wells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with biotinylated Estrone.
2. ***Standard (Standard 0-6),*** 7 vials, 1 mL each, ready to use;
   Concentrations: 0, 10.0, 20.0, 30.0, 60.0, 120.0, 300.0 pg/mL
   Conversion: pg/mL x 3.69 = pmol/L
   Contain non-mercury preservative.
3. ***Control Low & High***, 2 vials, 1 mL each, ready to use;
   For control values and ranges please refer to vial label or QC-Datasheet.
   Contain non-mercury preservative.
4. ***Enzyme Conjugate***, 1 vial, 14 mL, ready to use,
   Sheep anti-Estrone antibody conjugated to horseradish peroxidase.
   Contains non-mercury preservative.
5. ***Substrate Solution***, 1 vial, 25 mL, ready to use,
   Tetramethylbenzidine (TMB).
6. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5 M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
7. ***Wash Solution***, 1 vial, 30 mL (40X concentrated),
   see “Preparation of Reagents”

Note: *Sample Diluent* for sample dilution is available upon request.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 ± 10 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Scale paper or semi-logarithmic graph paper or software for data reduction
- Pipettes 100-1000 µL
- Centrifuge capable of 3000 - 2000 xg
- Plate shaker capable of ~ 600 rpm

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

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

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200mL.

The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, ALPCO 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 official regulations.

5 SAMPLE 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 sample, 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 <u>not</u> be used in the assay.

5.1 Sample Collection

Saliva samples should be collected only using special saliva sampling devices.

Due to the cyclic secretion pattern of steroid hormones, proper timing of the sampling is important. In order to avoid arbitrary results, it is recommended that 5 samples always be taken within a period of 2 – 3 hours (*multiple sampling*). Food may contain significant amounts of steroid hormones thus samples preferably should be taken while fasting. If fasting is a problem, the collection period should be timed just before lunch or before dinner (preferably before a meal).

5.2 Sample Storage and Preparation

Saliva samples in general are stable at ambient temperatures for several days. Mailing of such samples by ordinary mail without cooling will not create a problem.

The saliva samples may be stored at 2 °C to 8 °C for up to one week, and should be frozen at –20 °C for longer periods; repeated thawing and freezing is not a problem.

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 should be frozen in the deep freeze at least overnight. The next morning, the frozen samples are warmed up to room temperature and mixed carefully.

Then the samples are to be centrifuged for 5 to 10 minutes (at 2000 - 3000 xg). Now the clear colorless supernatant is easily pipetted.

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.

5.3 Sample Dilution

If in an initial assay, a sample is found to contain more than the highest standard, the samples can be diluted with *Sample Diluent* and re-assayed as described in Assay Procedure.

For the calculation of the concentrations the dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL sample + 90 µL *Sample Diluent* (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (mix thoroughly)

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and samples 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 disposable 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.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of microtiter wells in the frame holder.
2. Dispense **100 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL *Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have complete mixing in this step.
4. Incubate for **60 minutes** at room temperature, on a plate shaker (~ 600 rpm).
5. Briskly shake out the contents of the wells.
   Rinse the wells **4 times** with diluted *Wash Solution* (400 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 **200 µL** of ***Substrate Solution*** to each well.

7. Incubate for **15 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
9. Determine 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*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using scale paper or semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with the 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 or reported as >300.0 pg/mL. For the calculation of the concentrations the dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generation at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 pg/mL)	2.78
Standard 1 (10.0 pg/mL)	2.71
Standard 2 (20.0 pg/mL)	2.54
Standard 3 (30.0 pg/mL)	2.04
Standard 4 (60.0 pg/mL)	1.31
Standard 5 (120.0 pg/mL)	0.58
Standard 6 (300.0 pg/mL)	0.22

7. 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 insure the day to day validity of results.

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 to ensure the accuracy of the results.

Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not correspond to the established acceptable ranges of the control material, 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 ALPCO directly.

8 ASSAY CHARACTERISTICS

8.1 Assay Dynamic Range

The range of the assay is between 4.7 pg/mL – 300 pg/mL.

8.2 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay:

Substance	Cross-reactivity (%)
Progesterone	0.0
Testosterone	0.1
17-OHP	0.3
DHEA	2.7
Cortisol	0.0
Estradiol	13.5

9 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

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

The sample donor should not eat, drink, chew gum or brush teeth for 30 minutes before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

9.1 Interfering Substances

Visible blood contamination in saliva samples will affect results.

9.2 Drug Interferences

No substances (drugs) are currently known to us that have an influence on the measurement of Estrone in a sample.

9.3 High-Dose-Hook Effect

No hook effect was observed in this test.

10 LEGAL ASPECTS

10.1 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 ALPCO.

10.2 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 are 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.

11 REFERENCES

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