Product information

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Useŕs Manual

Estrone free in Saliva ELISA

REF **IB79312**

Σ **96 wells**

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

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Please use only the valid version of the Instructions for Use provided with the kit.

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1 INTRODUCTION

1.1 Intended Use

The **IBL-AMERICA Estrone free in Saliva ELISA** is an enzyme immunoassay for the precise determination of free Estrone in saliva. **For Research Use Only – Not for Use in Diagnostic Procedures**.

2 PRINCIPLE OF THE TEST

The IBL-AMERICA Salivary Estrone ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **principle of competitive binding**. The microtiter wells are coated with a polyclonal (rabbit) antibody directed towards a unique antigenic site of the estrone molecule. Endogenous estrone of a sample competes with an estrone-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 estrone in the sample. Having added the substrate solution, the intensity of colour developed is inversely proportional to the concentration of estrone in the sample.

3 WARNINGS AND PRECAUTIONS

- This kit is for research use only.
- 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.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C - 8 °C in the sealed foil pouch and used in the frame provided.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- 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.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- 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.
- Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
- Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
- Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- 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.
- 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.
- Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- 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.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

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- For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from IBL-America.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with estrone antibody (polyclonal).
2. **CAL 0 – 5 Standard (Standard 0 - 5)**, 6 vials, 1 mL each, ready to use;
   Concentrations: 0 - 3 - 12.3 - 37 - 111 - 333 pg/mL;
   Conversion: pg/mL × 3.69 = pmol/L;
   The standards are calibrated against the following reference material: Estrone solution (Certified Reference Material; E-075; Cerilliant)
   Contain non-mercury preservative.
3. **CONTROL low & high Control Low & High**, 2 vials, 1 mL each, ready to use; For control values and ranges please refer to QC-Datasheet. Contain non-mercury preservative.
4. **SAM DIL Sample Diluent**, 1 vial, 3 mL, ready to use, Contains non-mercury preservative.
5. **ENZ CONJ Enzyme Conjugate**, 1 vial, 14 mL, ready to use, Estrone conjugated to horseradish peroxidase; contains non-mercury preservative.
6. **SUB TMB Substrate Solution**, 1 vial, 25 mL, ready to use, Tetramethylbenzidine (TMB).
7. **STOP SOLN 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.
8. **WASH SOLN 40x Wash Solution**, 1 vial, 30 mL (40X concentrated), See “Reagent Preparation“.

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

4.2 Materials required but not provided

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

4.3 Storage Conditions

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.
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 1200 mL. *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 the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-America 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.

5 SAMPLE COLLECTION AND PREPARATION

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 subject should discard the sample, rinse the sampling device with water, wait for 10 minutes and take a new sample.

5.1 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 Diagnostics 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. 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.

5.2 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.

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 reassayed as described in Assay Procedure. For the calculation of the concentrations this 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 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.

6.2 Assay 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. Incubate for **30 minutes** at room temperature.
4. Dispense **100 µL Enzyme Conjugate** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **30 minutes** at room temperature.
6. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with **400** µL diluted Wash Solution 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!
7. Add **150 µL** of **Substrate Solution** to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µL** of **Stop Solution** to each well.
10. 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 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 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 Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) 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 > 333 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

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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 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 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 IBL-America directly.

8 PERFORMANCE CHARACTERISTICS

8.1 Assay Dynamic Range

The range of the assay is between 0.12 pg/mL - 333.0 pg/mL.

8.2 Specificity of Antibodies (Cross Reactivity)

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

Compound	Spiked concentration (pg/mL)	% Cross-reactivity
Estrone 3-sulfate	250	52.88
Estradiol	100	8.65
Estriol	1000	0.32
Progesterone	2400	0.08
17-OH Progesterone	1000	0.05
DHEA-S	1000	0.15
Androstenedione	1000	0.09
4-Androstene-3,17-dione	250	1.66
Cortisol	30000	ND
DHEA	1440	ND
Testosterone	1000	ND
Cortisone	250	1.06
Tetrahydrocortisone	1000	0.23
Ethisterone	250	0.32

ND = none detected (< 0.08 pg/mL)

8.3 Sensitivity

The analytical sensitivity of the IBL-America ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard 0 and was found to be 0.12 pg/mL

The Limit of Blank (LoB) is 0.08 pg/mL.
The Limit of Detection (LoD) is 1.073 pg/mL.
The Limit of Quantification (LoQ) is 3.104 pg/mL.

8.4 Reproducibility

8.4.1 Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (pg/mL)	CV (%)
1	10	9.28	8.5
2	10	37.85	9.4
3	10	59.17	2.4
4	10	126.80	8.8

8.4.2 Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (pg/mL)	CV (%)
1	30	9.64	14.1
2	30	38.57	8.2
3	30	56.58	4.2
4	30	131.11	7.1

8.5 Recovery

Recovery of the IBL-America ELISA was determined by adding increasing amounts of the analyte to 4 different samples containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) were assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (pg/mL)		33.60	49.19	107.21	298.68
Average Recovery (%)		97.7	97.2	107.8	96.6
Range of Recovery (%)	from	86.6	86.3	102.9	94.0
	to	107.2	110.6	112.2	97.7

8.6 Linearity

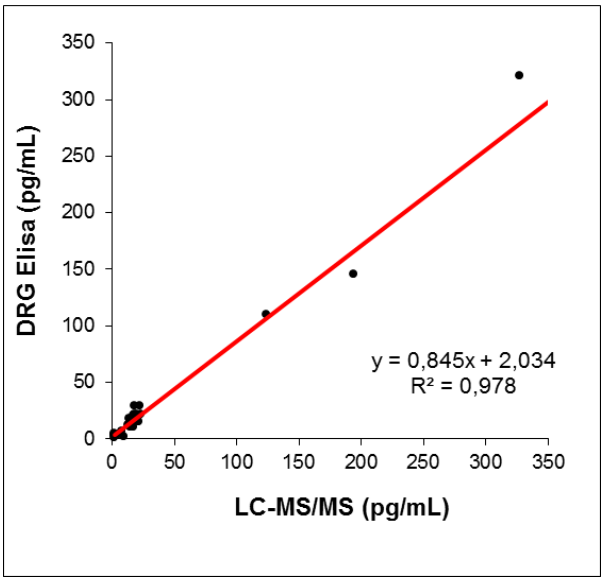
4 samples containing different amounts of analyte were serially diluted with *Sample Diluent*. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

		Sample 1	Sample 2	Sample 3	Sample 4
Concentration (pg/mL)		66.00	100.00	122.21	174.27
Average Recovery (%)		98.5	96.4	106.7	99.5
Range of Recovery (%)	from	93.9	92.8	97.8	88.5
	to	109.1	104.0	113.9	108.9

8.7 Comparison Studies

A comparison of IBL-America Salivary Estrone ELISA (y) and the Reference Method LC-MS/MS (x) using samples gave the following correlation:

n = 26
r = 0.989
y = 0.845x + 2.034



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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 instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

9.1 Interfering Substances

Visible blood contamination in saliva samples will affect results.

9.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to 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 lab 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 IBL-AMERICA.

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. 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 / LITERATURE

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SYMBOLS USED WITH IBL-AMERICA ELISAS

Symbol	English	Deutsch	Français	Español	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità

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