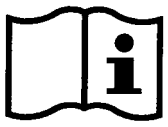
Product information



User´s Manual

Estriol free
in Saliva ELISA

REF **IB79315**

Σ **96 Wells**

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

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1 INTRODUCTION

1.1 Intended Use

The **IBL-AMERICA Estriol free in Saliva ELISA** is an enzyme immunoassay for the precise measurement of free estriol in saliva. **For research use only, not for use in diagnostic procedures.**

1.2 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.

2 PRINCIPLE

The IBL-America Estriol free in Saliva 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 antibody. Endogenous unconjugated (“free”) Estriol of a 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 color developed is inversely proportional to the concentration of free Estriol in the sample.

3 WARNINGS AND PRECAUTIONS

1. For Research Use Only – Not for use in diagnostic procedures
2. 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.
3. 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
11. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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.

16. Avoid contact with Stop Solution. It may cause skin irritation and burns.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material 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 Microtiterplate**, 12 x 8 (break apart) strips with 96 wells;
   Wells coated with an anti-Estriol antibody
2. **CAL | 0-5 Calibrator (Calibrator 0-5),** 6 vials, 1 ml each, ready to use;
   Concentrations: 0 – 2.5 – 15 – 100 – 600 – 4000 pg/ml
   Conversion: Estriol (pg/ml) x 3.5 = pmol/l
3. **CONTROL | 1-2 Control low / Control high**, 2 vials, 1.0 ml each, ready to use;
   For control values and ranges please refer to QC-Datasheet
4. **ENZ | CONJ | 100x Enzyme Conjugate concentrate**, 1 vial, 0.5 ml (100X concentrated); see "Preparation of reagents"; Estriol conjugated to horseradish peroxidase
5. **DIL | BUF Enzyme Conjugate Dilution Buffer**, 1 vial, 30 ml, ready to use;
6. **SUB | TMB Substrate Solution**, 1 vial, 22 ml, ready to use;
   contains tetramethylbenzidine (TMB)
7. **STOP | SOLN Stop Solution**, 1 vial, 7 ml, ready to use;
   contains 2 N Hydrochloric Acid solution
8. **WASH | SOLN | 10x Wash Solution**, 1 vial, 50 ml (10X concentrated);
   see "Preparation of Reagents"

Note: Additional *Calibrator 0* for sample dilution is available upon request.

4.2 Materials required but not provided

- Microcentrifuge
- A microtiterplate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes (10 µl, 50 µl, 100 µl, 200 µl).
- Microplate mixer operating more than 600 rpm
- Vortex mixer
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage conditions

When stored at 2°C to 8°C unopened reagents will be stable 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. Take care that the foil bag is sealed tightly.

4.4 Reagent preparation

Allow the reagents and the required number of wells to reach room temperature (21-26°C) before starting the test.

Wash Solution:
Dilute 50 ml of 10X concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml.
The diluted Wash Solution is stable for at least 3 months at room temperature (21-26°C).

Enzyme Conjugate:
Dilute the 100X concentrated *Enzyme Conjugate* with *Dilution Buffer*, i.e. 0.1 ml *Enzyme Conjugate concentrate* to a final volume of 10 ml with *Enzyme Conjugate Dilution Buffer*. Mix gently.
Stability of the prepared Enzyme-Conjugate: Stable for 3 hours at 21°C - 26°C
Prepare immediately before use.

4.5 Disposal of the kits

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 Sheet.

4.6 Damaged test kits

In case of any severe damage of the test kit or components, IBL-AMERICA has to be informed in writing, 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 individual should discard the sample, rinse the sampling device with water, wait for 10 minutes and take a new sample. Do not chew anything during the sampling period. Any pressure on the teeth may result in falsely elevated measurements due to an elevated content of gingival liquid in the saliva 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 may 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.

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 be 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 slightly 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 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.

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5.3 Sample Dilution

Samples expected to contain estriol concentrations higher than the highest calibrator should be diluted with the zero calibrator before performing the assay. The additional dilution step has to be taken into account for the calculation of the result.

Example:

a) Dilution 1:10: 10 µl saliva + 90 µl Calibrator 0 (mix thoroughly)
b) Dilution 1:100: 10 µl of dilution a) + 90 µl Calibrator 0 (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.
- Respect the incubation times as stated in this instructions for use.

6.2 Assay procedure

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators, controls and samples.
2. Dispense **50 µl** of each **calibrator, control and sample** with new disposable tips into appropriate wells.
3. Dispense **100 µl** of diluted **Enzyme Conjugate** into each well.
4. Incubate for **60 minutes** at room temperature on a Microplate shaker.

   Important Note:
   Optimal reaction in this assay is markedly dependent on shaking of the microplate!
5. Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by striking the microplate on absorbent paper.
6. Add **200 µl** of **Substrate Solution** to each well.
7. Incubate without shaking for **30 minutes** in the dark.
8. Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
9. Determine the absorbance of each well at 450±10 nm. It is recommended to read the wells within 15 minutes.

6.3 Results

1. Calculate the average absorbance values for each set of calibrators, controls and samples.
2. Using 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 calibration 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 calculation method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

Conversion to SI units:

Estriol (pg/ml) x 3.5 = pmol/l

7 QUALITY CONTROL

Good laboratory practice requires that controls should 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 national 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 at 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 Analytical Sensitivity

The lowest analytical detectable level of Estriol that can be distinguished from the Zero Calibrator is 1.4 pg/ml at the 2SD confidence limit.

8.2 Specificity (Cross Reactivity)

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Estriol.

Steroid	% Cross reaction
Androstenedione	< 0.1
Cortisol	< 0.1
11-Desoxycortisol	< 0.1
Estradiol	0.1
Estrone	< 0.1
Pregnenolone	< 0.1
Prednisolon	< 0.1
Prednison	< 0.1
Progesterone	< 0.1
Testosterone	< 0.1

8.3 Assay dynamic range

The range of the assay is between 2.5 – 4,000 pg/ml.

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8.4 Reproducibility

8.4.1 Intra-Assay

The intra-assay variation was determined by 15 replicate measurements of 3 saliva samples within one run. The within-assay variability is shown below:

Mean (pg/ml)	79.3	307.3	850.7
SD	7.4	24.4	69.8
CV (%)	9.4	7.9	8.2
n =	15	15	15

8.4.2 Inter-Assay

The inter-assay (between-run) variation was determined by duplicate measurements of 3 saliva samples in 10 different tests.

Mean (pg/ml)	63.2	393.3	553.1
SD	5.4	19.1	35.9
CV (%)	8.6	4.8	6.5
n =	10	10	10

8.5 Recovery

Using the Calibrator Matrix spiking solutions were prepared. Aliquots of these solutions were spiked into 500 µl of three saliva samples. All samples were measured by the IBL-America Estriol free in Saliva assay.

Sample	**Spiking (pg/ml)**	**Measured (pg/ml)**	**Expected (pg/ml)**	**Recovery (%)**
1	-	14.9	-	-
	550	575.6	565	102%
	600	673.6	615	109%
	700	734.6	715	103%
2	-	2.2	-	-
	550	447.8	552	81%
	600	626.1	602	104%
	700	746.5	702	106%
3	-	10.4	-	-
	500	408	510	80%
	600	516.5	610	85%
	700	700.4	710	99%

9 LIMITATIONS OF PROCEDURE

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

Blood contamination in saliva samples will affect results, and usually can be seen by eye.

9.2 Drug Interferences

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

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 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

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SYMBOLS USED WITH IBL-AMERICA ELISA

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consultez le Mode d'emploi	Consulte las Instrucciones	Consulti le istruzioni
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	Diagnostica in vitro
REF	Catalogue number	Katalog-Nr.	Référence	No de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	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	Temperature 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	Distributtore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Pocillos de la Microplaca	Micropozzetti
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de paro	Soluzione d´ arresto
Zero Standard	Zero Standard	Nullstandard	Standard 0	Standard 0	Standard zero
Standard	Standard	Standard	Standard	Calibrador	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué		Diluente del tracciante

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Symbol	Portugues	Dansk	Svenska	Ελληνικά
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Instruções de uso	Brugermanual	Användar manual	Εγχειρίδιο χρήστη
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til ”n” test	Innehåller tillräckligt till “n” tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..
Microtiterwells	Alvéolos de microtitulação	Mikrotiterbrønde	Brunnar i Mikrotiterplatta	Πηγαδάκια Μικροτιλοδοτήσεως
Enzyme Conjugate	Conjugado enzimático	Enzymkonjugat	Enzymkonjugat	Συζευγμένο ενζυμο
Substrate Solution	Solução de substrato	Substratopløsning	Substratlösning	Διάλυμα υποστρώματος
Stop Solution	Solução de paragem	Stopopløsning	Stopp lösning	Διάλυμα τερματισμού
Zero Standard	Padrão zero	Standard 0	Standard 0	Πρότυπο Μηδέν
Standard	Calibrador	Standard	Standard	Πρότυπα
Control	Controlo	Kontrol	Kontroll	Έλεγχος
Wash Solution	Solução de lavagem	Vaskebuffer	Tvätt lösning	Διάλυμα πλύσεως
Conjugate Diluent				

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