Product information

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Useŕs Manual

Estriol total ELISA

IVD

REF **IB79124**

Σ **96 wells**

Manufactured for:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Suite P, Minneapolis, MN 55432
Toll Free: (888) 523-1246 Fax: (763) 780-2988
www.ibl-america.com / info@ibl-america.com

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1 INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of total estriol concentration in human serum or plasma. Total Estriol ELISA is intended for laboratory use only.

1.1 Clinical Significance

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 rapid conjugation in the liver followed by urinary excretion with a half-life of ~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 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

Total 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 blue color that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is inversely proportional to the total estriol concentration in the sample.
Total Estriol concentration in the sample is calculated based on a series of standards.

3 REAGENT, MATERIAL AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. **CAL 0 – 4** Total Estriol **Standards** CAL 0 – CAL 4 (5 vials, 1 mL each)
2. **CONTROL 1 & 2** Total Estriol **Controls** (2 vials, 1 mL each); Control 1 and Control 2; Concentration of controls are indicated on the Certificate of Analysis
3. **ENZ CONJ Enzyme Conjugate** (1 vial, 22 mL); Estriol conjugated with horseradish peroxidase (HRP)
4. **SORB MT Coated Microplate** (1 breakable microplate); Anti-Estriol antibody adsorbed on microplate
5. **SUB TMB TMB Substrate Solution** (1 vial, 15 mL); H_2O_2-TMB 0.26 g/L, (avoid any skin contact)
6. **STOP SOLN Stop Solution** (1 vial, 15 mL); Sulphuric acid 0.15 mol/L, (avoid any skin contact)
7. **WASH SOLN 10x Wash Solution,** 10X Conc. (2 vials, 25 mL); Phosphate buffer 0.2 M pH 7.4

3.2 Reagents necessary not supplied

- Distilled water

3.3 Auxiliary materials and instrumentation

- Automatic dispenser
- Microplate reader (450 nm, 620-630 nm)

Note
Store all reagents between 2-8 °C in the dark. Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close immediately after use; once opened, it is stable until expiry date of the kit. Do not remove the adhesive sheet from the unused strips.

4 WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Proclin 300 as preservatives. 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 Total Estriol from 2 ng/mL to 200 ng/mL.
- The clinical significance of Estriol determination can be invalidated if the patient 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 °C - 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 specimens to reach room temperature (22 °C - 28 °C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials 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.
  To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- 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 lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6 PROCEDURE

6.1 Preparation of the Standard and Controls

The standards are ready to use and have the following concentration of Estriol:

	CAL 0	CAL 1	CAL 2	CAL 3	CAL 4
ng/mL	0	2	20	80	200

The Controls are ready to use. Once opened, Standards and Controls are stable for 6 months at 2-8 °C.

6.2 Preparation of Wash Solution

Dilute the content of each vial of the "Wash Solution 10X Conc." with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8 °C. In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.3 Preparation of the Sample

The determination of total Estriol should be performed in human serum or plasma.
Store samples at -20 °C if the determination is not performed on the same day of sample collection.
Avoid repetitive freezing and thawing of samples.

6.4 Procedure

Allow all reagents to reach room temperature (22-28 °C) for at least 30 minutes.
At the end of the assay, store immediately the reagents at 2-8 °C, avoid long exposure to room temperature. 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 (CAL0 - CAL4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample / Control	Blank
Sample / Control		20 µL	
Calibrator CAL 0-CAL 4	20 µL		
Conjugate	200 µL	200 µL	
Incubate at 37 °C for 1 hour. Remove the content from each well; wash the wells 3 times with 300 µL of diluted wash solution. **Important note:** during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer:** if you use automated equipment, wash the wells at least 5 times.			
TMB Substrate	100 µL	100 µL	100 µL
Incubate at 22 °C - 28 °C for 15 minutes in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake gently the microplate. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7 QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Total 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 standard 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 absorbances (Em) for each point of the standard curve (CAL 0 – CAL 4) and of each sample

8.2 Standard Curve

Plot the values of absorbance (Em) of the standards (CAL 0 – CAL 4) against concentration. Draw the best-fit curve through the plotted points (e.g: Four Parameter Logistic).

8.3 Calculation of Results

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

9 REFERENCE VALUE

Serum concentrations of Estriol are included in the following ranges:

weeks	Median	Range (ng/mL)
17°	18.0	(10 - 27)
18°	25.9	(14 - 51)
19°	39.5	(26 - 52)
20°	40.0	(27 - 53)
21°	45.6	(24 - 66)
22°	39.2	(25 - 58)
23°	56.1	(27 - 70)
24°	56.3	(28 - 75)
25°	64.3	(29 - 84)
26°	68	(41 - 105)
27°	57.4	(41 - 110)
28°	78.0	(38 - 127)
29°	87	(45 - 146)
30°	75	(45 - 160)
31°	88.0	(50 - 170)
32°	90.5	(46 - 175)
33°	100	(60 - 180)
34°	105.6	(60- 190)
35°	114.2	(65 - 200)
36°	126.0	(74 - 210)
37°	177.0	(90 - 234)
38°	190.0	(101 - 288)
39°	190.0	(102 - 306)
40°	180.0	(60 - 325)
41°	177.5	(95 - 280)

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10 PERFORMANCE AND CHARACTERISTICS

10.1 Precision

10.1.1 Intra Assay Variation

Within run variation was determined by replicate (16x) the measurement of three different sera in one assay. The within assay variability is ≤ 9.9%.

10.1.2 Inter Assay Variation

Between run variation was determined by replicate (10x) the measurement of three different sera in different lots of kit. The between assay variability is ≤10.3%.

10.2 Accuracy

The recovery of 5.5 - 11 - 22 - 44 ng/mL of Estriol gave an average value (±SD) of 103.02% ± 4.45% with reference to the original concentrations. The dilution test performed on three sera diluted 2 and 4 times gave an average value (±SD) of 107.86% ± 3.50%

10.3 Sensitivity

The lowest detectable concentration of Total Estriol that can be distinguished from the Standard 0 is 1.05 ng/mL at the 95% confidence limit.

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10.4 Specificity: cross reagent

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

Cross-reagent	Cross reactivity (%)
Estriol	100 %
16 epi-estriol	10.5 %
15 αOH-estriol	7.0 %
Estriol 3 Sulphate	2.0 %
Estradiol	0.1 %
17 epi-estriol	$< 1 \times 10^{-2}$ %
Estriol 3α Glucoronate	$< 1 \times 10^{-2}$ %
Estriol 16α Glucoronate	$< 1 \times 10^{-2}$ %
Estrone	$< 1 \times 10^{-4}$ %

10.5 Specificity: interfering substances

Interference by Bilirubin (conjugated and unconjugated), Hemoglobin and Triglycerides has been investigated on Estriol Total ELISA kit:

Substance	Assayed Conc.	Interference
Bilirubin (conjugated)	0.2 mg/mL	No
Bilirubin (unconjugated)	0.2 mg/mL	No
Hemoglobin	2 mg/mL	No
Triglycerides	6 mg/mL	No

No interference has been observed with the substances under investigation; following good laboratory practices, it is anyway advised to avoid to use highly lipaemic or haemolysed samples.

10.6 Specificity: plasma and SST tube

Interference in plasma and SST (serum separation tube) samples has been evaluated. Serum obtained from the same patient has been used as reference.

Sample	Interference
SST (serum separation tube)	No
EDTA plasma	No
Lithium heparin plasma	No
Sodium heparin plasma	No

No interference has been observed.

10.7 Correlation

The new IBL-America Estriol Total ELISA kit was compared to the old IBL-America Estriol Total ELISA kit. 35 serum samples were analysed.
The linear regression curve was calculated:

$y = 1.02x - 1.88$
$r^2 = 0.969$

11 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

12 BIBLIOGRAPHY

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13 TROUBLESHOOTING

ERRORS / POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

too high within-run CV%

- reagents and/or strips not pre-warmed to room temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run CV %

- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

SYMBOLS USED WITH IBL-AMERICA ASSAYS

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	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	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
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore