DATA SHEET

LUCIO®-Medical ELISA

Total *Estriol*

REF ELI-5582

IVD

Version 1.0

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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* kit is intended for laboratory use only.

2. Introduction

Estriol (also o*Estriol*) 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..

3. Principle of the Test

Total *Estriol* (antigen) in the sample competes with horseradish-peroxidase *Estriol* (enzyme-labelled-antigen) for binding onto the limited number of anti *Estriol* (antibody) sites on the *microplates* (solid phase).
After incubation, the bound/free separation is performed by a simple solid-phase washing.
The enzyme 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.
Total *Estriol* concentration in the sample is calculated based on a series of standard.
The colour intensity is inversely proportional to the Total *Estriol* concentration in the sample.

4. Reagents and Materials Supplied

1) Total *Estriol* ***Standards*** (4 vials, 1 mL each)
2) Total *Estriol* ***Control*** (1 vial, 1 mL)
   Concentration of control is Lot-specific and is indicated on Quality Control Report
3) ***Incubation Buffer*** (1 vial, 30 mL)
   Phosphate buffer 50 mM pH 7.5; BSA 1 g/L, stabiliser
4) ***Enzyme Conjugate*** (1 vial, 1 mL)
   Estriol conjugated with horseradish peroxidase (HRP)
5) ***Coated Microplate*** (1 breakable microplate)
   Anti-*Estriol* IgG adsorbed on microplate
6) ***TMB Substrate Solution*** (1 vial, 15 mL)
   H_2O_2-TMB 0.26 g/L, (avoid any skin contact)
7) ***Stop Solution*** (1 vial, 15 mL)
   Sulphuric acid 0.15 mol/L, (avoid any skin contact)

5. Additional Required Materials

- Distilled water.
- Automatic dispenser.
- Microplates reader (450 nm)

6. Storage & Stability

Store all reagents between 2 °C - 8 °C in the dark.
Open the bag of the Coated Microplate only when it is at room temperature and close immediately after use.
Do not remove the adhesive sheet from the unused strips.

7. Warnings and Precautions

- This kit is intended for *in vitro* use by professional persons only.
- Use appropriate personal protective equipment while working with the reagents provided.
- 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.
- 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 syntetic steroids.
- Please adhere strictly to the sequence of pipetting steps provided in this protocol.
- All reagents should be stored refrigerated at 2 °C - 8 °C in their original container. Any exceptions are clearly 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 is your 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.
- 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 should not be used in the assay. Highly lipemeic or haemolysed specimens should similarly not be used
- Plate readers measure vertically. Do not touch the bottom of the wells.

Waste Management

Reagents must be disposed off in accordance with local regulations.

8. Specimen Collection and Preparation

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.

9. Procedure of the Test

I. Preparation of the Standard (S1,S2,S3,S4)

Before use, mix for 2 minutes.
The s*tandards* have the following concentration of *Estriol*:

	S1	S2	S3	S4
ng/mL	2.0	20.0	80.0	200.0

Stable until the expiry date of the kit at 2 °C - 8 °C.
Once opened, the standards are stable for 6 months at 2 °C - 8 °C.

II. Preparation of diluted Conjugate

Prepare immediately before use.
Add 10 µL *Conjugate* (reagent 4) to 2.0 mL of *Incubation Buffer*.
Mix gently for 5 minutes, with a rotating mixer.
Stable for 3 hours at room temperature (22 °C – 28 °C).

III. Test Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each of the four points of the standard curve (S1-S4), two for B_o and for each sample, two for *Control* and one for Blank.

Reagent	B_0	Standard	Sample/ Control	Blank
Incubation buffer	**20 µL**			
Sample/*Control*			**20 µL**	
Standard S1-S4		**20 µL**		
Diluted conjugate	**200 µL**	**200 µL**	**200 µL**	
Incubate at 37 °C for 1 hour. Remove the content from each well; wash the wells 2 times with **300 µL** of distilled water.				
TMB Substrate	**100 µL**	**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**	**100 µL**
Shake gently the *microplate.* Read the absorbance (E) at 450 nm against Blank.				

10. Interpretation of the Results

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

I. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample

II. Standard Curve

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

III. 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.

IV. Reference Values

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)**

V. 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

11. 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.

12. Performance Characteristics

I. Precision

Intra Assay Variation

Within run variation was determined by replicate measurements (16x) of two different control sera in one assay.
The within assay variability is ≤ 9.7 %.

Inter Assay Variation

Between run variation was determined by replicate measurements (12x) of three different control sera in different kit lots. The between assay variability is ≤ 10 %.

II. Accuracy

The recovery of 10 – 40 – 100 ng/mL of Total *Estriol* added to two samples gave an average value (±SD) of 94.88 % ± 4.47 % with reference to the original concentrations.

III. Sensitivity

The lowest detectable concentration of Total *Estriol* that can be distinguished from the zero standard is 0.22 ng/mL at the 95 % confidence limit.

IV. Specificity

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

Estriol	100 %
16 epi-*Estriol*	10.5 %
15 αOH-*Estriol*	7.0 %
Estriol 3 Sulphate	2.0 %
Estradiol	0.1 %
17 epi-*Estriol*	< 1 x 10-2 %
Estriol 3α Glucoronate	< 1 x 10-2 %
Estriol 16α Glucoronate	< 1 x 10-2 %
Estrone	< 1 x 10-4 %

V. Correlation with RIA

The LUCIO®-Medical ELISA Total *Estriol* was compared to another commercially available Total *Estriol* assay. 32 serum samples were analysed.
The linear regression curve was calculated

y = 0.86 x + 3.85
r2 = 0.952
y = LUCIO®-Medical ELISA Total *Estriol* (ELI-5582)
x = Total *Estriol* Adaltis RIA Kit

13. References

1) Fischer-Rasmussen, W., et al Acta Obstet Gynecol. Scand. 60-417 - 420 (1981)
2) Truran, P.L., et al Clin. Chem. 28/12, 2393 (1982)
3) Vining, R. F., et al J. Clin. Endoc. Metab. 56, 454 (1983)
4) Bagger, P.V, et al Acta Obstet Gynecol Scand 60, 187 (1981)
5) Osterman, T.M, et al Clin. Chem. 25(5) 716 (1979)
6) Wisdom, G.B. Clin. Chem. 22 (8) 1243-1255 (1976)

Symbol	English	Deutsch	Français	Nederlands	Español	Italiano
	Consult instructions for use	Gebrauchsan-weisung beachten	Consulter les instructions d'utilisation	Gebruiksaanwij-zing	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conform Europese richtlijnen	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	In vitro diagnostisch gebruik	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Catalogus nummer	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Lot nummer	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Geschikt voor <n> tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungs-temperatur	Température de conservation	Opslag temperatuur	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthalt-barkeitsdatum	Date limite d'utilisation	Houdbaarheids datum	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabrikant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distributeur	Distribuidor	Distributore
Cont.	Content	Inhalt	Conditionnement	Inhoud	Contenido	Contenuto

Symbol	Polski	Suomi	Portugues	Dansk	Svenska	Ελληνικά
	Patrz: ulotka informacyjna	Katso käyttöohjeet	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisning en	Εγχειρίδιο χρήστη
CE	Znak zgodności CE	CE-merkitty	Conformidade com as normas europeias	Europaeisk overensstemm else	Europeisk överensstämm else	Ευρωπαϊκή Συμμόρφωση
IVD	Tylko do użytku in-vitro	In vitro-diagnostiikka	Diagnóstico in vitro	In vitro diagnostik	In vitro diagnostik	in vitro διαγνωστικό
REF	Numer katalogowy	Luettelo-numero	Catálogo n.º	Katalognummer	Katalognummer	Αριθμός καταλόγου
LOT	Numer serii	Eränumero	No do lote	Lot nummer	Batchnummer	Αριθμός Παρτίδος
	Wystarszajonce na "n" powturzeń	Sisältää tarvikkeet "n" testiin	Contéudo suficiente para <n> testes	Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura przechowywani a	Säilytys-lämpötila	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemp ratur	Θερμοκρασία αποθήκευσης
	Data ważności	Viimeinen käyttöpäivä	Prazo de validade	Udløbsdato	Utgångsdatum	Ημερομηνία λήξης
	Producent	Valmistaja	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by	Dystrybutor	Tukkumyyjä	Distribuidor	Distributør	Distributör	Διανομέας
Cont.	Zawartość	Sisältö	Conteúdo	Indhold	Innehåll	Περιεχόμενο

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