



DCM019-6
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TOTAL ESTRIOL ELISA

for routine analysis

Direct immunoenzymatic determination of Total Estriol in human serum or plasma

IVD



LOT

See external label

2°C - 8°C



Σ = 96 tests

REF DKO019

INTENDED USE

The Eagle Biosciences [Total Estriol ELISA Assay Kit](#) utilizes a competitive immunoenzymatic colorimetric method for quantitative determination of Total Estriol concentration in human serum or plasma. Total Estriol ELISA Assay Kit is intended for research use only and not to be used in diagnostic procedures.

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

In this Total Estriol ELISA Assay Kit, the 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 (H₂O₂) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H₂SO₄) is added. The color intensity is inversely proportional to the Total Estriol

concentration in the sample. Total Estriol concentration in the sample is calculated based on a serie of standards.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

- Total Estriol Calibrators (4 vials, 1 mL each)
CAL1 **REF** DCE002/1907-0
CAL2 **REF** DCE002/1908-0
CAL3 **REF** DCE002/1909-0
CAL4 **REF** DCE002/1910-0
- Total Estriol Control (1 vial, 1 mL)
Concentration of Control is lot-specific and is indicated on the Certificate of Analysis **REF** DCE045/1903-0
- Incubation Buffer (1 vial, 30 mL)
Phosphate buffer 50 mM pH 7.5; BSA 1 g/L; stabilisers
REF DCE001-0
- Conjugate (1 vial, 1 mL)
Estriol conjugated with horseradish peroxidase (HRP)
REF DCE002/1902-0
- Coated Microplate (1 breakable microplate)
Anti estriol antibody adsorbed on microplate
REF DCE002/1903-0
- TMB Substrate (1 vial, 15 mL)
H₂O₂-TMB 0.26 g/L (*avoid any skin contact*)
REF DCE004-0
- Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L (*avoid any skin contact*)
REF DCE005-0

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm)

Note

Store all reagents between 2-8°C in the dark.

Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use.

Do not remove the adhesive sheet from the unused strips.

4. WARNINGS

- This Total Estriol ELISA Assay Kit is intended for research 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.
- Some reagents of the Total Estriol ELISA Assay Kit contain small amounts of Proclin 300^R 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/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.
- This Total Estriol ELISA Assay Kit 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 in the Total Estriol ELISA Assay Kit should be stored refrigerated at 2-8°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 Total Estriol ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange Total Estriol ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels 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.
- 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 Calibrators (C₁...C₄)

Before using, mix for 2 minutes.

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

	C ₁	C ₂	C ₃	C ₄
ng/mL	2.0	20.0	80.0	200.0

Stable until the expiry date of the kit at 2-8°C.

Once opened, the Calibrators are stable for 6 months at 2-8°C.

6.2. Preparation of Diluted Conjugate

Prepare immediately before use.

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

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 the sample connection. Avoid repetitive freezing and thawing of samples.

The Control is ready for use.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C).**
- 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 B₀, two for each point of the calibration curve (C₁-C₄), two for each Control, two for each sample, one for Blank

Reagent	B ₀	Calibrator	Sample/ Control	Blank
Incubation buffer	20 µL			
Sample/ Control			20 µL	
Calibrator C ₁ -C ₄		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 300µL of distilled water.				
TMB Substrate	100 µL	100 µL	100 µL	100µL
Incubate at 22±28°C for 15 minutes in the dark.				
Stop Solution	100µL	100µL	100µL	100µL
Shake gently the microplate. Read the absorbance (E) at 450 nm against Blank.				

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 calibration 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 absorbance (E_m) of B₀ point, of each point of the calibration curve (C₁-C₄) and of each sample

8.2. Calibration curve

Plot the values of absorbance of B₀ and of the Calibrators against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

8.3. Calculation of Results

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

9. REFERENCE VALUES

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)

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

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

10.1.2. Inter Assay Variation

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

10.2. Accuracy

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

10.3. Sensitivity

The lowest detectable concentration of Total Estriol that can be distinguished from the B₀ is 0.22 ng/mL at the 95 % confidence limit.

10.4. 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 α OH-estriol	7.0 %
Estriol 3 Sulphate	2.0 %
Estradiol	0.1 %
17 epi-estriol	$< 1 \times 10^{-2}$ %
Estriol 3 α -Glucoronate	$< 1 \times 10^{-2}$ %
Estriol 16 α -Glucoronate	$< 1 \times 10^{-2}$ %
Estrone	$< 1 \times 10^{-4}$ %

10.5. Correlation with RIA

The Diametra Total Estriol ELISA was compared to another commercially available Total Estriol assay. 32 serum samples were analysed.

The linear regression curve was calculated:

$$y = 0.86 x + 3.85$$

$$r^2 = 0.952$$

y = Total Estriol Diametra Elisa Kit

x = Totale Estriol Adaltis RIA Kit

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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ERROR 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
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation