AccuDiag™ Free Estriol ELISA Kit

Cat# 3171-17

IVD See external Label 2°C 8°C 96 Tests

Test	Free Estriol
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Immunosorbent
Detection Range	.15-40 ng/ml
Sample	25 µL
Total Time	~ 75 min.
Shelf Life	12-14 Months from the manufacturing date
Specificity	100 %
Sensitivity	0.03 ng /ml

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Free Estriol concentration in serum and plasma.

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 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.

TEST PRINCIPLE

Free 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 substrate (H_2O_2) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined.
Free Estriol concentration in the sample is calculated based on a series of standard.

The colour intensity is inversely proportional to the Free Estriol concentration in the sample. Free Estriol concentration in the sample is calculated through a Standards curve.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Calibrators (7 vials, 1 mL each)**

S0	REF DAS0/3171-17
S1	REF DAS1/3171-17
S2	REF DAS2/3171-17
S3	REF DAS3/3171-17
S4	REF DAS4/3171-17
S5	REF DAS5/3171-17
S6	REF DAS6/3171-17

2. **Control (1 vial, 1 mL)**
See concentration of Control on the Certificate of Analysis REF **CTRL (Range mentioned on COA)**

3. **Conjugate (1 vial, 15 mL, ready to use)**
Estriol conjugated with Horseradish peroxidase (HRP) REF **DA-C/3171-17**

4. **Coated Microplate (1 breakable microplate)**
Anti-Estriol antibody adsorbed on microplate REF **DA-P3171-17**

5. **TMB Substrate (1 vial, 15 mL)**
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)* REF **DA-T/3171-17**

6. **Stop Solution (1 vial, 15 mL)**
Sulphuric acid 0.15 mol/L *(avoid any skin contact)* REF **DA-S/3171-17**

7. **10X Conc. Wash Solution (1 vial, 50 mL)**
Phosphate buffer 0.2M, Proclin < 0,0015% REF **DA-W/3171-17**

Materials required but not provided

1. Distilled water.
2. Automatic dispenser.
3. Microplates 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 it immediately after use.

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.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
- Some reagents contain small amounts of Sodium Azide or Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.

- 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 Free Estriol from 0.15 ng/mL to 40 ng/mL.
- The clinical significance of Free Estriol determination can be invalidated if the patient was treated with natural or syntetic steroids.

ASSAY PROCEDURE

Preparation of the Standards (S_0…S_6)

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

	S_0	S_1	S_2	S_3	S_4	S_5	S_6
ng/mL	0	0.15	0.75	2	5	15	40

The Standards are stable until the expiry date printed on the label. Once opened, the Calibrators are stable six months at 2-8°C.

Preparation of the Sample

The determination of Estriol can be performed in human plasma as well as in serum. 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.

Preparation of Wash Solution

Dilute the contents of each vial of "10X Conc. Wash Solution" 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.

Procedure

1. **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.
2. Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
3. To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
4. 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 Standards curve (S_0-S_6), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Standards S_0-S_6	**25 µL**		
Sample/ Control		**25 µL**	
Conjugate	**100 µL**	**100 µL**	
Incubate at 37°C for 1 hour. Remove the contents from each well; wash the wells 6 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.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22-28°C) for 15 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

RESULTS

Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the Standards curve (S_0-S_6) and of each sample.

Standards curve

Plot the values of absorbance (Em) of the Standards (S_0-S_6) against concentration. Draw the best-fit curve through the plotted points (es: Four Parameter Logistic).

Calculation of Results

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

PERFORMANCE CHARACTERISTICS

a. Precision

Intra Assay Variation
Within run variation was determined by replicate the measurements (20x) of three different sera in one assay. The within assay variability is ≤ 7.4%.

Inter Assay Variation
Between run variation was determined by replicate the measurements (10x) of three different sera in different lots of kit. The between assay variability is ≤ 7.7%.

b. Accuracy

The recovery of 0.51 - 1.02 - 2.03 - 4.07 - 8.13 ng/mL of Estriol added to a sample gave an average value (±SD) of 96.96% ± 6.61% with reference to the original concentrations.

c. Sensitivity

The lowest detectable concentration of free estriol that can be distinguished from the Calibrator 0 is 0.03 ng/mL at the 95% confidence limit.

d. Specificity

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

Free Estriol	100 %
16 epi-estriol	10.5 %
15 αOH-estriol	7.0 %
Estriol 3 Sulphate	2.0 %
Estradiol	0.1 %
17 epi-estriol	< $1x10^{-2}$ %
Estriol 3 α-Glucoronate	< $1x10^{-2}$ %
Estriol 16 α-Glucoronate	< $1x10^{-2}$ %
Estrone	< $1x10^{-4}$ %

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Correlation

Free Estriol ELISA was compared to another commercially available Free Estriol assay. 80 serum samples were analysed.

The linear regression curve was calculated:
y = 0.90 x + 0.15
r^2 = 0.819

Waste Management

Reagents must be disposed off in accordance with local regulations.

EXPECTED VALUES

47 samples from not pregnancy women were assayed with Diagnostic Automation, Inc. Free Estriol ELISA kit; the median of the values is:

N°	Median (Estriol)
47	0.08 ng/mL

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 Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of 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.

PRECAUTIONS

1. 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.
2. All reagents 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.
3. Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
4. Do not interchange 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.
5. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
6. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
7. 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
8. to repeat the dose response curve in each plate
9. 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.
10. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
11. Maximum precision is required for reconstitution and dispensation of the reagents.
12. Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
13. Plate readers measure vertically. Do not touch the bottom of the wells.

REFERENCES

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ISO 13485
ISO 9001

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