DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com

IVD	See external label	2°C-8°C	Σ=96 tests	REF Cat # 3171Z

Free Estriol

Cat # 3171Z

Test	**Free Estriol**
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**Peroxidase – Conjugated Competitive ELISA**
Detection Range	**1- 12 ng/ml**
Sample	**25ul serum**
Specificity	**100%**
Sensitivity	**.05ng/ml**
Total Time	**~75 min**
Shelf Life	**12-14 months**

* Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.

INTENDED USE

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

CLINICAL SIGNIFICANCE

Diagnostic Automation 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.

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-Sustrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbance 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.

REAGENT, MATERIAL AND INSTRUMENTATION

Reagent and material supplied in the kit

1. Free Estriol Standards 4x (1 vial = 1 mL)

STD_1 REF **DAS1/3171Z**
STD_2 REF **DAS2/3171Z**
STD_3 REF **DAS3/3171Z**
STD_4 REF **DAS4/3171Z**

2. Incubation buffer (1 bottle) 30 mL

Phosphate buffer 50 mM pH 7.4; BSA 1 gr/L
REF **DA-I/3171Z**

3. Conjugate (1 bottle) 1.0 mL

Estriol-HRP conjugate REF **DA-C/3171Z**

4. Coated Microplate (1 microplate breakable)

Anti-Estriol IgG adsorbed on microplate
REF **DA-P/3171Z**

5. TMB-substrate (1 bottle) 15 mL

H_2O_2-TMB 0.25gr/L
(avoid any eye contact) REF **DA-T/3171Z**

6. Stop solution (1 bottle) 15 mL

Sulphuric acid 0.15 mol/L
(avoid any eye contact) REF **DA-S/3171Z**

Reagents necessary not supplied
Distilled water.
Auxiliary materials and instrumentation
Automatic dispenser.
Microplates reader

Note
Store all reagents between +2÷8C° in the dark.
Open the bag of reagent 3 (Coated Microplate) only when it is at room temperature and close immediately after use.
Do not remove the adhesive sheet from the unused strips.

PRECAUTION

- Do not use heavily hemolized samples.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants.
- This method allows the determination of Free Estriol from 0.1 ng/mL to 12.0 ng/mL.
- For concentration of Free Estriol over 12 ng/mL dilute the serum (1 + 3) with Incubation Buffer
- Considere the diluting factor when calculating the results.
- The clinical significance of Free Estriol determination can be invalidated if the patient was treated with natural or syntetic steroids.

PROCEDURE

1. Preparation of the Standard (S_1,S_2,S_3,S_4)
The standard has the following concentration of Estriol:

	S_1	S_2	S_3	S_4
ng/ml	0.1	1.0	4.0	12.0

Stability: until the expiration date printed on the kit.

Once open, the standards are stable six months at +4°C.

2. Preparation of Diluted Conjugate
Prepare immediately before use.
Add 10 µl stock solution (reagent 2) to 2.0 mL of Incubation Buffer (reagent 1).
Mix gently for 5 minutes, with rotating mixer
Stable for 3 hours at room temperature.

3. Preparation of the Sample
The determination of Estriol can be performed in plasma as well as in serum .
Store reagent at -20°C if the determination is not performed on the same day of the sample connection.

PROCEDURE
As it is necessary to perform the determination in duplicate, prepare two wells for each of the four points of the standard curve (S_1-S_4), two for B_o and for each sample, one for Blank.

Reagent	B_0	Standard	Sample	Blank
Incubation buffer	20 µL			
Sample			20 µL	
Standard S1-S4		20 µL		
Diluted conjugate	200 µl	200 µl	200 µl	
Incubate at 37°C for *1 hour* Remove the contents from each well; wash the wells with 300µL of distilled water. Repeat the washing procedure by draining the water completely				
TMB-substrate	100µL	100µL	100µL	100µL
Incubate at 22-28°C for *15 minutes* in the dark.				
Stop solution	100µL	100µL	100µL	100µL
Read the absorbance (E) at 450 nm against Blank.				

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 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.

LIMITATION OF PROCEDURE

1. Assay Performance

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or haemolysed specimen(s) should similarly not be used. 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 one plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

2. Interpretation

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.

RESULTS

1. Mean Absorbance

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

2. Standard Curve

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

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.

REFERENCE VALUE

Free Estriol

Pregnancy weeks	Serum or Plasma (ng/mL) Median	Range
14°	0.6	(0.2 - 3.0)
15°	0.8	(0.2 - 3.5)
16°	1.0	(0.3 - 4.2)
17°	1.2	(0.4 - 5.2)
18°	1.4	(0.4 - 5.8)
19°	1.6	(0.4 - 6.2)
20°	2.0	(0.4 - 6.8)
22°	2.5	(0.4 - 9.1)
24°	2.7	(0.4 - 9.1)
26°	4.0	(1.9 - 9.5)
28°	5.0	(2.2 - 10.1)
30°	5.0	(2.0 - 10.8)
32°	5.6	(2.5 - 11.3)
34°	5.8	(2.2.- 12.7)
36°	9.0	(2.5 - 25.0)
37°	10.6	(3.6 - 25.3)
38°	15.1	(6.6.- 29.7)
39°	13.7	(6.7.- 25.3)
40°	14.8	(7.2 - 22.9)
41°	17.4	(8.8 - 31.5)

PERFORMANCE AND CHARACTERISTICS

Precision

1. Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 4.8%.

2. Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 8.8%.

Accuracy

The recovery of 1 – 2 – 4 – 8 ng/mL of Estriol added to a sample gave an average value (±SD) of 101.6% ± 6.8% with reference to the original concentrations.

Sensitivity
The lowest detectable concentration of estriol that can be distinguished from the zero standard is 0.05 ng/ml at the 95 % confidence limit.

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 αGlucoronate	$< 1x10^{-2}\%$
Estriol 16 αGlucoronate	$< 1x10^{-2}\%$
Estrone	$< 1x10^{-4}\%$

Correlation with RIA
The Diagnostic Automation Inc. Free Estriol ELISA was compared to another commercially available Cortisol assay. Serum samples of 33 females and 30 males were analysed according in both test systems.
The linear regression curve was calculated
$y = 0.958\,x + 0.05$
$r = 0.995$ ($r^2 = 0.99$)
Hook Effect
The Free Estriol ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 40ng/ml

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Tel: (818) 591-3030 Fax: (818) 591-8383

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