



DIAGNOSTIC AUTOMATION, INC.

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IVD	See external label	2°C-8°C	Σ=96 tests	REF #3171-17
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ESTRIOL FREE

Direct immunoenzymatic determination of estriol free on serum or plasma.

Cat. No. 3171-17

1. PRINCIPLE

Free Estriol (antigen) in the sample competes with horseradish peroxidase-Estriol (enzyme-labeled 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 (H₂O₂) and the TMB-Substrate (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbance are determined.

Estriol concentration in the sample is calculated based on a series of standard.*

The color intensity is inversely proportional to the Estriol concentration of in the sample.

2. REAGENT, MATERIAL AND INSTRUMENTATION

2.1 Reagent and material supplied in the kit

- * S₁-S₂-S₃-S₄ (1 bottle each) 1 mL
Estriol Standards
- 1. Incubation buffer (1 bottle) 30 mL
Phosphate buffer 50 mM pH 7.4; BSA 1 gr/L
- 2. Conjugate (1 bottle) 0.4 mL
Estriol-HRP conjugate
- 3. Coated Microplate (1 microplate breakable)
Anti-Estriol IgG adsorbed on microplate
- 4. TMB-Substrate (1 bottle) 12 mL
HydrogenPeroxide/TMB 0.25gr/L (avoid any skin contact)
- 6. Stop solution (1 bottle) 12 mL
Sulphuric acid 0.15 mol/L(corrosive:avoid any skin contact)

2.2 Notes

Store all reagents between +2 and + 8°C 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 sheets on the strips until used

2.3 Reagents necessary which are not supplied with the kit

Distilled water.

2.4 Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader

2.5 Preparation of reagents

*. Standard (S₁,S₂,S₃,S₄) (liquid)

Before use, mix for 2 min. with rotating mixer

The standards has the following concentration of Estriol:

	S ₁	S ₂	S ₃	S ₄
ng/ml	0.1	1.0	4.0	12.0

Stable until the expiration date of kit at +4°C,

1. Diluted Conjugate . (Prepare immediately before use)

Add 10 □l stock solution (reagent 2) to 2.0 mL of Incubation Buffer (reagent 1).
Mix gently.

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.

3.1 Precaution

- Do not use heavily hemolyzed samples.
 - Maximum precision is required for reconstitution and dispensation of the reagents.
 - This method allows the determination of Estriol from 0.1 ng/mL to 12.0 ng/mL.
 - For concentration of Estriol over 12 ng/mL dilute the serum (1 + 3) with saline solution.
- Consider the diluting factor when calculating the results.
-The clinical significance of Estriol determination can be invalidated if the patient was treated with natural or synthetic steroids.

4. 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 B₀ and for each sample, one for Blank.

Pipette:

	B ₀	Standard	Sample	Blank
Reagent 1	20 □l	-----	-----	-----
Sample	-----	-----	20 □l	-----
Standards S ₁ -S ₄	-----	20 □l	-----	-----
Diluted Conjugate	200 □l	200 □l	200 □l	-----

Incubate at 37°C for 1 hour

Remove the contents from each well; wash the wells with 300 □L of distilled water.Repeat the washing procedure by draining the water completely.

Pipette:

	B ₀	Standard	Sample	Blank
TMB-Substrate	100 □l	100 □l	100 □l	100 □l

Incubate at 20-25°C for 15 minutes in the dark.

Pipette:

	B ₀	Standard	Sample	Blank
Stop solution	100 \square l	100 \square l	100 \square l	100 \square l

Read the absorbance (E) at 450 nm against Blank.

5. STANDARD CURVE - CALCULATION OF RESULTS

5.1 Mean absorbance and relative percentage

Calculate the mean of the absorbances (E_m) corresponding to the single points to the standard curve (S₁-S₄), and of each sample. Express data as the percentage of the mean absorbance of B₀ (EmB₀) with the following formula:

$$(B/B_0)\% = \frac{E_m}{(E_m B_0)} \times 100$$

5.2 Standard curve

Plot the values of the standards (S₁-S₄) expressed as (B/B₀)% on the enclosed logit-log paper. Extrapolate the line passing through the points.

5.3 Calculation of results

Interpolate the values of the samples expressed as (B/B₀)% on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

6. REFERENCE VALUES

“unconjugated 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)

7. PERFORMANCE CHARACTERISTICS

7.1 Specificity

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

estriol	100.0 %
16-epi-estriol	10.5 %
15 \square -OH-estriol	7.0 %
estriol - 3 - sulphate	2.0 %
estradiol	0.1 %
17-epi-estriol	<1x10 ⁻² %
estriol-3 \square glucuronate	<1x10 ⁻² %
estriol- 16 \square glucuronate	<1x10 ⁻² %
estrone	<1x10 ⁻⁴ %

7.2 Sensitivity

The sensitivity of this method, calculated as two times the S.D. from B₀, is 2 pg when the value of (B/B₀)% is approx 90%.

7.3 Precision

The inter and intra-run precision had a coefficient of variation of 3.6% and .6.1% respectively.

7.4 Accuracy

The recovery of 0.1 - 1.0 - 4.0 - 12.0 ng/mL of Estriol added to “plasma-free” sample gave an average value (\pm SE) of 94 % \pm 4.0% with reference to the original concentrations.

7.5 Correlation with RIA

Correlation with RIA performed on the same samples:

$$y = 0.90 + 1.12x$$

$$r = 0.996$$

$$n = 25$$

$$p < 0.001$$

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