17β-ESTRADIOL ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 6107605

CE

Enzymatic Immunoassay for the Determination of 17β-Estradiol in Human Serum

For In Vitro Diagnostic Use Only

Store at 2 to 8°C.

PRINCIPLE OF THE TEST

17β-Estradiol (antigen) in the sample compets with horseradish-peroxidase 17β-Estradiol (enzyme-labeled-antigen) for binding onto the limited number of anti 17β-Estradiol (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 chromogen (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbance are determinated.

17β-Estradiol concentration in the sample is calculated based on a series of standard.

The color intensity is inversely proportional to the 17β-Estradiol concentration in the sample.

REAGENTS

Materials provided with the kit:

17 β-Estradiol Standards
* S_0-S_1-S_2-S_3-S_4-S_5 (1 bottle each)

1. Conjugate (1 bottle) 22 mL **ready-to-use**
   17 β-Estradiol-HRP conjugate
2. Coated Microplate (1 microplate breakable)
   Anti-17 β-Estradiol IgG adsorbed on microplate
3. Conc. Wash Solution 10X (1 bottle = 50 mL)
   Phosphate buffer 0,2 M, proclin < 0,002%
4. TMB-Substrate (1 bottle) 15 mL
   H_2O_2-TMB 0.25 gr/L (avoid any skin contact)
5. Stop Solution (1 bottle) 15 mL
   Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact)

2.2 Notes

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

Open the bag of reagent 2 (Coated Microplate)) only when it is at room temperature and close immediately after use.

Do not remove the adhesive sheet from the inutilized strips.

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 (S_0,S_1,S_2,S_3,S_4 S_5) (liquid)
The standard has the following concentration of 17β-Estradiol:

	S_0	S_1	S_2	S_3	S_4	S_5
pg/mL	0	20	120	300	600	2000

Stability: until the expiration date printed on the kit. When are open, the standards are stable six month at 4°C.

Preparation of Wash Solution
Dilute the content of the vial "Conc. Wash Solution 10X" 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 complete dissolution of crystals, for greater accuracy dilute the whole bottle of concentrated wash solution to 500mL on taking care also transfer crystals with washing of the bottle, then mix until crystals are completely dissolved.

PREPARATION OF THE SAMPLE

The determination of 17β-Estradiol can be performed in plasma as well as in serum of patients who have observed fast.

Store reagent at -20°C if the determination is not performed on the same day of the sample collection

PRECAUTION

Do not use heavily hemolized samples.

Maximum precision is required for reconstitution and dispensation of the reagents.

This method allows the determination of 17β-Estradiol from 20 pg/mL to 2000 pg/mL.

The clinical significance of the determination 17β-Estradiol can be invalidated if the patient was treated with cortisone or natural or syntetic steroids.

ASSAY PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the six points of the standard curve (S_0-S_5), two for each sample, one for Blank.

1. Pipette:

	Standard	Sample	Blank	
Sample	---	25 µL	---	
Standards S_0 –S_5	25 µL	---		---
Conjugate	100 µL	100 µL	---	

2. Incubate at *+37°C* for **2 hours**

3. Remove the contents from each well. Wash the wells with **300 µL** of distilled water.

4. Repeat the washing procedure by draining the water completely.

5. Pipette:

	Standard	Sample	Blank
TMB-Substrate	100 µL	100 µL	100 µL

6. Incubate at room temperature (20-25°C) for **30 minutes** in the dark.

7. Pipette:

	Standard	Sample	Blank
Stop Solution	100 µL	100 µL	100 µL

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

STANDARD CURVE - CALCULATION OF RESULTS

Mean absorbance and relative percentage
Calculate the mean of the absorbances (Em) corresponding to the single points to the standard curve and of each sample. Express data as the percentage of the mean absorbance of B_0 (EmB$_0$) with the following formula:

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

Standard curve
Plot the values of the standards expressed as $(B/B_0)\%$ on the enclosed logit-log paper.
Extrapolate the line passing through the points.

Calculation of results
Interpolate the values of the samples expressed as $(B/B_0)\%$ on the standard curve to obtain the corresponding values of the concentrations expressed in pg/mL.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population.

The serum or plasma 17β-Estradiol value are comprised in the following intervals:

WOMAN	Follicular phase	30 - 100 pg/mL
	Ovulatory peak	130 - 350 pg/mL
	Luteinic phase	50 - 180 pg/mL
	Menopause	< 60 pg/mL
MAN		< 40 pg/mL
CHILDREN		< 60 pg/mL

PERFORMANCE CHARACTERISTICS

Specificity

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

17β-Estradiol	100.0 %
Estrone	2.0 %
Estriol	0.39 %
Testosterone	0.02 %
Cortisol	7×10^{-3} %
Progesterone	3×10^{-4} %
Dehydroepiandrosterone	1×10^{-4} %

Sensitivity

The sensitivity of this method, calculated as two times the S.D. from B_0, is 5 pg when the value of $(B/B_0)\%$ is approx 96.5%.

Precision

The inter and intra-run precision had a coefficient of variation of 3.2% and 5.4% respectively.

Accuracy

The recovery of 0, 20, 120, 300, 600, 2000 pg/mL of 17β-Estradiol added to "plasma-free" sample gave an average value (±SE) of 96.7% ± 3.1% with reference to the original concentrations.

Correlation with RIA
Correlation with RIA performed on the same samples:

$y = 7.63 + 0.979x$
$r = 0.991$
$n = 32$
$p < 0.001$

REFERENCES

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