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User's Manual

Free Estriol ELISA kit

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Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Free Estriol ELISA Kit is an enzyme immunoassay for the quantitative measurement of free estriol (unconjugated estriol) in serum during the second half of pregnancy.

General Description

Estriol (E3) is the major estrogen formed by the fetoplacental unit during pregnancy. Unconjugated E3 passes through the placenta into the maternal circulation, where it is rapidly converted into glucuronide and sulfate derivatives to facilitate its excretion. The half-life of estriol in the maternal bloodstream is only 20-30 minutes. Its measurement, therefore offers a convenient and quick evaluation of current fetal status. Plasma estriol levels increase steadily throughout pregnancy and most rapidly during the third trimester (28-40 weeks). A sudden decrease in fetoplacental E3 production will result in a rapid fall in unconjugated E3 in the maternal serum. There are several potential advantages to measuring unconjugated E3 rather than total serum or urinary E3. Unconjugated estriol levels are free from effects related to maternal renal or hepatic disease, and are not altered by the administration of certain antibiotics. Unconjugated E3 more accurately reflects fetal outcome in diabetic pregnancies - and since no hydrolysis of unconjugated E3 is required, a more rapid tumaround for the test result is possible.

Principles of Testing

The Free Estriol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal [rabbit] antibody directed towards an antigenic site on the Estriol molecule. Endogenous Estriol of a patient sample competes with an Estriol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estriol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Estriol in the patient sample.

Reagents And Materials Provided

1. **Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-Estriol antibody (polyclonal).
2. **Standard** (Standard 0-5), 6 vials, 1 mL, ready to use; Concentrations: 0 - 0.3 - 1.2 - 4.0 - 15 - 40 ng/mL.
3. **Control Low & High**, 2 vials, 1 mL each, ready to use; For control values and ranges please refer to vial label or QC-Datasheet. Contain non-mercury preservative.
4. **Enzyme Conjugate**, 1 vial, 14 mL, ready to use, Estriol conjugated to horseradish peroxidase; Contains non-mercury preservative.
5. **Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
6. **Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.5 M H2SO4, Avoid contact with the stop solution. It may cause skin irritations and burns.
7. **Wash Solution**, 1 vial, 30 mL (40x concentrated).

Note: Additional Standard 0 for sample dilution is available upon request.

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450 ± 10 nm)
2. Calibrated variable precision micropipettes.
3. Absorbent paper.
4. Distilled or deionized water
5. Timer
6. Semi logarithmic graph paper or software for data reduction

Storage

2°C to 8°C

Specimen Collection And Preparation

Serum should be used in this assay. Do not use haemolytic, icteric or lipaemic specimens. Please note: Samples containing sodium azide should not be used in the assay.

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Specimens should be capped and may be stored for up to 4 days at 2°C to 8°C prior to assaying. Specimens held for a longer time (At least one year) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution:

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard 0 and reassayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Standard 0 (mix thoroughly).

Reconstitution And Storage

When stored at 2°C to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution

Add deionized water to the 40x concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.

Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **10 µL** of each **Standard**, **Control** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL Enzyme Conjugate** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature.
5. Briskly shake out the contents of the wells. Rinse the wells **4 times** with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

6. Add **100 µL** of **Substrate Solution** to each well.
7. Incubate for **30 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **100 µL** of **Stop Solution** to each well.
9. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the Stop Solution.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

Calculation

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method The results in the Instructions for Use have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as> 40 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard Optical Units (450 nm)

Standard 0 (0.0 ng/mL) 1.79

Standard 1 (0.3 ng/mL) 1.48

Standard 2 (1.2 ng/mL) 1.18

Standard 3 (4.0 ng/mL) 0.81

Standard 4 (15.0 ng/mL) 0.52

Standard 5 (40.0 ng/mL) 0.38

Performance Characteristics

Assay Dynamic Range

The range of the assay is between 0.075 - 40 ng/mL.

Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Added steroid	Concentration of steroid	OD 450	Measured concentration
Estriol (E3)	40 ng/mL	0.39	39.67 ng/mL
Testosterone	16 ng/mL	1.758	n.d.
Estradiol (E2)	2 ng/mL	1.579	n.d.
Estrone (E1)	2 ng/mL	1.712	n.d.
Cortisol	800 ng/mL	1.775	n.d.

n.d. = non detectable

Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard 0 and was found to be 0.075 ng/mL.

Reproducibility

Intra-Assay

The within-assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	2.1	4.7
2	20	6.2	3.2
3	20	14.6	3.0

Inter-Assay

The between-assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	12	2.1	4.6
2	12	5.7	8.5
3	12	13.3	9.5

Recovery

Recovery of this ELISA was determined by adding increasing amounts of the analyte to three different patient sera containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		1.3	3.6	7.8
Average Recovery		100.8	101.8	106.9
Range of Recovery [%]	from	89.0	92.3	98.5
	to	103.8	109.8	112.3

Linearity

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		2.6	7.2	15.6
Average Recovery		98.2	99.7	100.2
Range of Recovery [%]	from	86.3	90.4	96.4
	to	107.9	106.8	103.7

Interferences

Interfering Substances

Haemoglobin (up to 4 mg/mL), bilirubin (up to 0.125 mg/mL) and triglyceride (up to 30 mg/mL) have no influence on the assay results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Estriol in a sample.

Precautions

1. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H2SO4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before

reusing them. If inhaled, take the person to open air.

20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Limitations

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

References

1. Bashore, R.A., Westlake, J.R. Plasma unconjugated estriol values in high risk pregnancy. Am. J. Obstet. Gynecol., June 15, 1977, p371-380