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CALBIOTECH

Free Estriol Serum ELISA

Cat#. EF313S (96 tests)

INTENDED USE

The Calbiotech Inc. Estriol (E3) ELISA kit is used for the quantitative measurement of free Estriol (E3) in human serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

Estriol is one of the three main estrogens in humans and is produced in significant amounts during pregnancy by the placenta. This ELISA measures unbound estriol in serum for research on maternal–fetal hormone exchange, estrogen signaling pathways, and pregnancy-related endocrine physiology.

PRINCIPLE OF THE TEST

The Estriol (E3) kit is based on the principle of competitive binding between E3 in the test specimen and E3-HRP conjugate for a constant amount of Rabbit anti-E3 antibody. In the first incubation, goat anti-Rabbit IgG-coated wells are incubated with 25µl of E3 standards, patient samples, 50µl Estrone-HRP conjugate reagent and 50µl rabbit anti-E3 reagent, at room temperature, for 60 minutes at room temperature. During the incubation, HRP labeled E3 competes with the endogenous E3 in the standard and sample, for a fixed number of binding sites of the specific E3 antibody. Thus, the amount of E3 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E3 in the specimen increases. Unbound E3 peroxidase conjugate is then removed and the wells washed. Next, TMB Reagent is added and incubated at room temperature for 30minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is spectrophotometrically measured at 450nm. A standard curve is prepared relating color intensity to the concentration of E3.

MATERIALS PROVIDED		96 TESTS
1.	Microwells coated with Goat anti-Rabbit IgG	12x8x1
2.	Estriol Standard Set: 6 vials (ready to use)	6x1 mL
3.	Estriol Control Set: 2 vials (ready to use)	2x1 mL
4.	Estriol (E3) Enzyme Conjugate, 1 bottle (ready to use)	10 mL
5.	Rabbit Anti- Estriol Reagent, 1 bottle (ready to use)	10 mL
6.	TMB substrate, 2 bottles (ready to use)	2x8 mL
7.	Stop solution, 1 bottle (ready to use)	12 mL
8.	20X Wash concentrate, 2 bottles	2x25 mL

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. precision pipettes
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm
5. absorbance paper or paper towel
6. graph paper

STORAGE AND STABILITY

1. Store the kit at 2 – 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

PREPARATION OF REAGENTS

20X Wash Buffer: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. Once the test has been started, all steps should be completed without interruption.

1. Secure the desired number of microwells strips in the holder.
2. Dispense 25µl Estriol Standards, controls and samples into appropriate wells.
3. Dispense 50 µl Enzyme Conjugate into each well.
4. Dispense 50ul anti- Estriol reagent into each well.
5. Cover plate and incubate for 60minutes, at room temperature.
6. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted wash solution. Strike the wells sharply on absorbent paper to remove residual water droplets.
7. Add 100 µl of Substrate Solution into each well.
8. Cover plate and incubate for 30minutes at room temperature.
9. Stop the enzymatic reaction by adding 50 µl of Stop Solution into each well.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check Estriol standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached**.**
2. To construct the standard curve, plot the absorbance for free Estriol standards (vertical axis) versus standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

EXAMPLE OF A STANDARD CURVE

Estriol (ng/ml)	Absorbance (450 nm)
0	2.53
0.5	1.99
2.5	0.91
5	0.47
15	0.16
30	0.07

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.