

# CALDON BIOTECH, INC.

## Free Estriol ELISA

Catalog No. EF068S  
(96 tests)

### INTENDED USE

This Free Estriol ELISA kit is used for the quantitative measurement of free Estriol in human serum or plasma.

### SUMMARY AND EXPLANATION

Estriol (1,3,5(10)-estratriene-3,16 $\beta$ ,17 $\beta$ -triol; E3) is one of the three major naturally-occurring estrogens produced almost exclusively during pregnancy. Maternal Estriol levels, alone and in combination with hCG and AFP, have been recommended to monitor fetal status. 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 Estriol levels, reaching a late-gestational peak which is ~2-3 orders of magnitude greater than nonpregnant levels. In the maternal circulation, Estriol undergoes rapid conjugation in the liver followed by urinary excretion with a half-life of ~20 minutes. Therefore, maternal Estriol levels can provide a dynamic estimate of fetal production rates. In terms of estrogenic activity, Estriol is much less potent than Estradiol. Because Estriol concentrations are subject to diurnal and episodic variation, it is common practice to refer serum measurements to a baseline, defined for the patient as either the average or the highest of her three most recent Estriol results.

### PRINCIPLE OF THE TEST

This Free Estriol kit is a solid phases competitive ELISA. The samples, standards, and controls are incubated with Estriol enzyme conjugate on wells coated with anti-Estriol antibody. Free Estriol in the patient's serum competes with Estriol enzyme conjugate for binding sites. Unbound serum proteins and Estriol enzyme conjugate is washed off by washing with distilled water. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of free Estriol in the samples. A standard curve is prepared relating color intensity to the concentration of the free Estriol.

### MATERIALS PROVIDED

1. Microwells coated with anti-Estriol Ab (12x8x1 wells). Total of 96 wells.
2. Estriol Standard: 5 vials (0.4 mL each). Ready to use.
3. Enzyme Conjugate: One Bottle (12 mL). Ready to use
4. TMB Substrate reagent: One Bottle (12 mL). Ready to use.
5. Stop Solution: One Bottle (12 mL). Ready to use.

### 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

1. Potential biohazardous materials: The calibrator and controls 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 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. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. 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

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8° C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

### ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

- Place the desired number of coated strips into the holder
- Pipet 25  $\mu$ L of Estriol standards, control and patient's sera.
- Add 100  $\mu$ L of Estriol Enzyme Conjugate to all wells.
- Incubate for 60 minutes at room temperature (18-26° C).
- Remove liquid from all wells. Fill wells distilled water. Wash six times. Blot on absorbent paper towels.
- Add 100  $\mu$ L of TMB substrate to all wells.
- Incubate for 30 minutes at room temperature.
- Add 100  $\mu$ L of stop solution to all wells. Shake the plate gently to mix the solution.
- Read absorbance on ELISA Reader at 450 nm within 20 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.

### EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following

values for free Estriol may be used as initial guideline ranges only:

Population	(ng/mL)
1st. Trimester (Weeks 4-13)	Less than 0.5
2nd. Trimester (Weeks 14-28)	0.3 - 5
3rd. Trimester (Weeks 29-40)	1.5 - 14

### LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patients history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

### PERFORMANCE CHARACTERISTICS

#### 1. Precision

- a. Intra-Assay Precision was determined by assaying 16 replicates of each of three sera; normal, low and high.

Serum	No. of Replicates	Mean Ng/mL	Standard Deviation	Coefficient of Variation%
Normal	16	11.5	0.7	6.0
Low	16	5.4	0.4	7.4
High	16	2.7	0.2	7.4

- b. Inter assay Precision was determined by assaying duplicates of three serum pools in 10 separate runs, using a standard curve constructed for each run.

Serum	No. of Replicates	Mean Ng/mL	Standard Deviation	Coefficient of Variation%
Normal	10	10.2	0.8	7.8
Low	10	6.5	0.5	7.9
High	10	2.3	0.2	8.7

## 2. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean Ng/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.06	0.04	0.14 ng/mL

## REFERENCES

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