3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Free Estriol ELISA

Catalog Number **SE120051**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Estriol (1, 3, 5(10)-estratriene-3, 16α, 17β-triol; E_3) 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 non-pregnant 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.

The Free Estriol ELISA kit is based on the principle of competitive binding between estriol in the test specimen and estriol-HRP conjugate for a constant amount of rabbit anti-Estriol. In the first incubation, goat anti-rabbit IgG-coated wells are incubated with 50 µL of estriol standards or patient samples, 100 µL Estriol-HRP conjugate reagent and 50 µL rabbit anti-Estriol reagent, at room temperature, for 60 minutes. During the incubation, HRP labeled estriol competes with the endogenous estriol in the standard or sample, for a fixed number of binding sites of the specific Estriol antibody. Thus, the amount of Estriol peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of estriol in the specimen increases. Unbound estriol peroxidase conjugate is then removed and the wells washed.

Next, TMB Reagent is added and incubated at room temperature for 15 minutes, 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 450 nm. A standard curve is prepared relating color intensity to the concentration of estriol.

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

Components

Materials Provided	96 Tests
Microwells coated with Goat anti-rabbit IgG	12 x 8 x 1
Standard: 6 vials (ready to use)	0.5 mL
Estriol Enzyme Conjugate (ready to use)	12 mL
Rabbit Anti-Estriol Reagent (ready to use)	7 mL
TMB substrate (ready to use)	12 mL
Stop solution (ready to use)	12 mL
Wash Solution 20x Concentrated	25 mL

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Pecision pipettes
- Disposable pipette tips
- Microwell reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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.

20x Wash Buffer

Prepare 1x Wash Buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability

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

Procedure

Notes:

The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

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

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.

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 50 µL Estriol Standards, controls, and samples into appropriate wells.
3. Dispense 100 µL Enzyme Conjugate into each well.
4. Dispense 50 µL anti-Estriol reagent into each well. Mix gently for about 10seconds.
5. Incubate for 60 minutes, 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.
   Note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add 100 µL of Substrate Solution to each well.
8. Incubate for 15 minutes 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.

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 the value is checked on every kit.
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

Free Estriol (ng/mL)	Absorbance (450 nm)
0	2.37
0.4	1.85
2	1.28
5	0.96
15	0.55
30	0.37

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

Product profile:

Sensitivity

The sensitivity of the assay is 0.094 ng/mL. The sensitivity was determined by calculating the mean plus 2 SD of the standard zero point tested 24 times in the same run.

Correlation

A total of 60 samples were tested by this kit and a commercially available Estriol ELISA kit. The linear regression curve was calculated as:
Y = 0.941x – 0.019, r = 0.988

Precision

Intra-Assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	20	1.53	0.10	6.62
2	20	3.96	0.20	5.13
3	20	10.87	0.43	3.96

Inter-Assay

Serum	No. of Replicates per test	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	16	1.59	0.09	5.96
2	16	3.95	0.21	5.26
3	16	10.24	0.47	4.56

Linearity

Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4, and 1:8.Estriol values were calculated and results were corrected with the dilution factor.

Original Value		Percentage of Recovery		
Serum	(ng/mL)	1:2	1:4	1:8
1	14.11	107	104	93
2	12.88	111	102	91
3	7.96	97.5	90	110

Specificity

Analyte	**% Cross reactivity**
Estriol	100
Androstenedione	0.00028
Androsterone	0.00033
Trans-Dehydroandrosterone	0.00129
Cortisone	0.00003
Corticosterone	0.00171
Cortisol	0.00093
17β-Estradiol	0.0122
Estrone	0.00156
Progesterone	0.00000
Prednisone	0.00493
Testosterone	0.00678

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