Human Estriol (unconjugated) ELISA Kit

Enzyme Immunoassay for the quantification of human free Estriol (unconjugated Estriol) in serum during the second half of pregnancy

Catalog number: ARG80842

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INTRODUCTION

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.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A highly specific Estradiol antibody has been pre-coated onto a microtiter plate. Estradiol containing samples or standards and an Estradiol-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free Estradiol compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound

material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Estradiol is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Standards 0-5 (0, 0.3, 1.2, 4, 15, 40 ng/ml)	4 X 1 ml (ready to use)	4°C
Control Low	1 ml (ready to use)	4°C
Control High	1 ml (ready to use)	4°C
HRP-Estradiol conjugate	14 ml (ready to use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	14 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Store the unopened reagents at 2 - 8°C until expiration date. Once opened the reagents are stable for 2 month when stored at 2 – 8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.
- All kit reagents and specimens should be brought to room temperature (21-26°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- 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 colored. Do not pour reagents back into vials as reagent

contamination may occur.

- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Storage: Specimens may be stored for up to 4 days at 2°C to 8°C and at least 12 months while stored at-20°C. Samples should be frozen only once at-20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Note: Do not use haemolytic, icteric or lipaemic specimens.

Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash Solution is stable for 2 weeks at room temperature.
- Samples: If the initial assay found samples contain Estriol higher than the highest standard, the samples can be diluted with Standard 0 (S0) and then re-assay the samples. 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 1:10 diluted a) + 90 µL Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 10 µl of each Standard, Control and samples into appropriate wells.
3. Add 100 µl of HRP-Estradiol conjugate into each well.
4. Cover wells and incubate for 60 minutes at RT.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of

liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

6. Add 100 µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature in dark.
7. Add 100 µl of Stop Solution to each well.
8. Read the OD with a microplate reader at 450 nm immediately. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

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

2. Using linear 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.

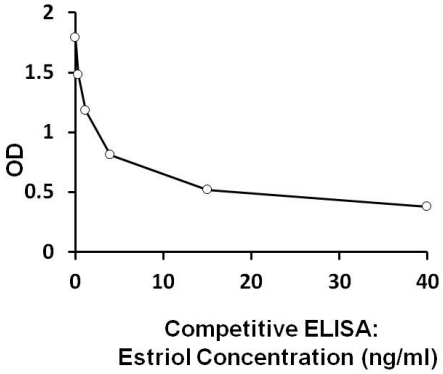
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

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.

EXAMPLE OF TYPICAL STANDARD CURVE

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



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 0.075 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.63% and inter-assay precision was 7.53%.

Specificity

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.

Recovery

89-112.3%