

## DE1612 Estriol, free/unconjugated ELISA

Technology	ELISA
Kit size	96 Tests
Sample material	serum
Sample preparation	-
Sample volume	10 $\mu$ l
Standard range	0.3 – 40 ng/ml
Incubation	60 min, 30 min at RT
Measuring system	TMB 450nm
Sensitivity	$\leq$ 0.02 ng/ml

**Special remarks:** 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 turnaround for the test result is possible.

The DEMEDITEC E3 EIA provides a direct non-isotopic method for measuring the total unconjugated estriol in unextracted human serum. This method uses a highly specific estriol antibody and an enzyme-labeled analyte. The colored end-product may be measured on a spectrophotometer. The long shelf life of this product, together with the elimination of radioisotopes, radiation counter and necessary licensing requirements make this method applicable to both large and small laboratories.

This assay cannot measure the free (unbound) Estriol in serum. In fact this cannot be achieved by any commercial immunoassay method. If there is a need for measuring the free estriol, then the best method is the measurement of salivary estriol. Demeditec is offering such a testkit in ELISA format with the catalogue number [DESLV3653](#).

This testkit coded DE1612 also can be used for veterinary investigations if the animal sample will be extracted using the following procedure:

## Extraction procedure for steroids

We are recommending an extraction procedure as follows.

1. Pipette 1 ml of Ethyl Acetate in a 2.5 ml PP microtube (e.g. from Sarstedt).
2. Add between 5 and 200  $\mu$ l of your sample.
3. Mix for 2-3 minutes on a vortexer.
4. Centrifuge the microtube briefly in order to achieve a clear separation of the 2 phases.
5. Deep freeze the microtube at  $-20^{\circ}\text{C}$  or lower for several hours (or overnight).
6. Decant the organic liquid phase into a new tube which can be used for an evaporation process (the specification of the new tube depends of the specifications of your evaporating device). Be sure that no frozen material will be decanted.
7. Evaporate the organic solvent in an appropriate device at elevated temperature. The boiling point of Ethyl Acetate is  $77^{\circ}\text{C}$ . Please stay clearly below this temperature while evaporating.
8. After evaporating the organic solvent until complete dryness re-dissolve your sample with a diluent. In most cases this will be the zero standard of the ELISA (or RIA) to be used for the quantification of the analyte.
9. The volume of the diluent to be used for the reconstitution depends of the sensitivity of your assay system in relation to the analyte concentration in the primary sample.
10. The dilution (or concentration) factor depends of the volumes you have used in #1 and #7.