

**PRIMATE ESTRADIOL ELISA TEST KIT****PRODUCT PROFILE AND INSTRUCTIONS****INTENDED USE**

The Microwell Estradiol EIA is an enzyme immunoassay system for quantitative determination of Estradiol levels in Primate serum/plasma samples. The test is intended for professional use as an aid in the monitoring of conditions related to serum Estradiol. The test kit is designed to be used by a trained, skilled professional only.

**INTRODUCTION**

Estradiol is a steroid hormone with a molecular weight of 272, secreted from the testis in the male and by the ovary in non-pregnant women. In circulation Estradiol primarily is bound to the sex hormone binding globulin (SHBG) and is present 1-2% as free hormone. Estradiol is metabolized in the liver to the more water soluble sulfate and glucuronide derivatives prior to excretion by the kidneys.

In women, the measurement of Estradiol is useful in evaluating the status of ovarian functions hypo-estrogen in case of delayed puberty, primary and secondary amenorrhea and menopause. Estradiol levels in normal adult females change throughout the normal menstrual cycle. In the early period of follicular development, the levels of Estradiol remain low. Approximately one week before the LH peak there is an initial decrease and then a more rapid rise of Estradiol, reaching a peak the day before the LH peak. Serum Estradiol can also be used as a measure of the maturity of the ovarian follicle.

In men, elevated levels of Estradiol are often associated with gynecomastia, tumor of testis or liver, or cirrhosis of the liver. Both male and female infants have high concentrations of Estradiol in cord blood. Concentrations in both sexes during the first week of life are lower in cord serum, but higher than in later childhood, corresponding to the increased amounts of LH and FSH in infants at this time. During the first three days of life the percentage of free Estradiol is very high, more than twice that of sexually mature women. In normal prepubertal children, Estradiol is less than 10pg/ml. During puberty Estradiol increase progressively in girls, in parallel with increases in gonadotropins. The importance of sequential Estradiol measurements for monitoring ovulation induction therapy, particularly in "in vitro" fertilization programs has recently been reported.

**PRINCIPLES OF TEST**

The E2 quantitative test is based on a solid-phase enzyme immunoassay based on competitive binding method. A sample (serum/ plasma/urine) containing an unknown amount of E2 to be assayed (unlabeled antigen) is added to a standard amount of a conjugated E2 (labeled antigen). The labeled and unlabeled antigens are then allowed to compete for high affinity binding sites on a limited number of antibodies coated on to the plate. After washing away the free antigen, the amount of labeled antigen in the sample is reversibly proportional to the concentration of the unlabeled antigen. The actual concentrations in unknown samples are obtained by means of a standard curve based on known concentrations of unlabeled antigen analyzed in parallel with the unknowns. After washing, substrate solution is added and the enzyme is allowed to react for a fixed time before the reaction is terminated. Absorbencies are measured at 405 nm using ELISA plate reader. A standard curve is produced using values from 5 standards from which absorbency values for blank tubes have been subtracted. Results for unknown may be read directly from this standard curve using either manual calculation or by a suitable computer program. This kit is suitable for the direct measurement of E2 in serum samples. It may also be used following an extraction procedure, for assaying urinary E2.

**Materials Provided**

1. Microtiter wells 96, coated with second antibody.
2. Enzyme Conjugate solution, 12 mL (Blue Solution)
3. E2 Standard Set: 0, 10, 30, 100, 300 and 1000, 5000 pg/mL.
4. Quality control set QC1, (80-100 pg/mL) and QC2, (600-800 pg/mL)
5. TMB Color Reagent, 12 mL
6. Stopping Solution (2N HCL), 6 mL
7. 20 X Wash Buffer, 20 mL (store at room temperature only).
8. Instructions

**Materials Required But Not Provided**

1. Semiautomatic pipettes: 20ul and 200ul
2. Disposable pipette tips
3. Microtiter plate shaker
4. Microtiter well reader.
5. Plate washer
6. Absorbant paper
7. 37 C incubator
8. Parafilm to cover plate
9. Distilled water

**PRECAUTIONS**

1. This kit contains reagents manufactured from Primate blood components. Nevertheless, all Primate blood products and samples should be considered potentially infectious and handling should be in accordance with the procedures defined by an appropriate biohazard safety guideline or regulations in your local and state.
2. The contents of this kit, and their residues, must not come into contact with ruminating animals or swine or other animals.
3. Avoid contact with the Stopping Reagent. It may cause skin irritation and burns.
4. Do not use reagents after expiration date.
5. Do not mix or use components from the kits with different lot numbers.
6. Replace caps on reagents immediately. Do not switch caps.
7. Reagents contain sodium azide (NaN<sub>3</sub>) as a preservative. On disposal, flush with a large volume of water to prevent azide build-up.
8. Do not pipette reagents by mouth.
9. Do not use reagents from other kits or mix with other manufactured test kits.

**STORAGE & STABILITY CONDITIONS**

1. Store the kit at 2-8 C upon receipt and when it is not in use. **Do not Freeze.**
2. Keep microtiter wells in a sealed bag with desiccants to minimize exposure to damp air.
3. Allow all the reagents to reach to room temperature before setting up the assay.
4. Remove only desired number of wells and seal the bag and store at 2-8 C as before.
5. Do not at any time mix or use components with other manufacturers kits. Do not use the kit components after expiration date and discard according to the state and local regulations.

**INSTRUMENTATION**

A microtiter well reader with bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 405 nm wavelength is acceptable for use in absorbency measurement.

**SPECIMEN COLLECTION AND PREPARATION**

1. This kit is suitable for use with serum or heparin plasma samples. The use of hemolytic or lipemic samples and samples with bilirubin will affect results and may interfere with the assay.
2. No special preparation of the samples is required. Avenous blood sample (enough to produce about 0.5 ml serum ) is collected aseptically.
3. If the sample is not tested immediately refrigerate at 2-8 C. If the storage period greater than 3 days are anticipated, the specimen should be frozen and repeated thawing and freezing should be avoided.
4. If the sample is turbid or contain precipitate may give false results. Such samples should be centrifuged before use.

**REAGENT PREPARATION**

1. Prepare Wash buffer by diluting 1 part with 19 parts of distilled water, excess amount may be stored at 2-8 C for couple of weeks.
2. Dilute highly concentrated specimen samples with Assay buffer and mix well before use in the assay.

## ASSAY PROCEDURE

### ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 25 ul of standards, samples, and QC controls into appropriate wells.
3. Add 100 ul of E2 Enzyme Conjugate Solution to each well (except those set for blanks),
4. Mix well for 30 sec. and incubate at 37C for 2 hours.
5. Discard the contents of the wells and wash the plate 5 times with Wash Solution (250-300ul) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture.
5. Add 100 ul TMB Substrate solutions into each well (including the blanks). Remember for pipetting order.
6. Incubate the plate for 20 minutes at room temperature.
7. Stop reaction by adding 50ul of Stopping Solution and gently mixed.
8. Read the absorbance at 450 nm with a micro well reader.

**NOTE:** The substrate incubation should be carried out within the temperature range 20-25C. For temperature outside this range, the duration of the incubation should be adjusted.

### URINARY ESTRADIOL MEASUREMENT

As an alternative to the normal direct assay method, urinary E2 may be assayed using the following extraction method (standards provided with the kit should not be extracted).

1. Using an automatic reagent dispenser, add 4.0 ml dichloromethane to 400 ul of urine sample.
2. Vortex mix for 30 seconds in pulses of about 5 seconds. Allow layer to separate.
3. Discard the upper aqueous layer.
4. Transfer 1000 ul of dichloromethane phase with a positive displacement pipette to another tube and evaporate to dryness in a fume hood.
5. Redissolve the residue in 200ul of sample dilution buffer, vortex mix 15 seconds, and again just before pipetting.
6. Run the assay according to the details of the procedure given above using 25ul and 50 ul aliquots of the extracted samples.

### CALCULATION OF RESULTS

1. Calculate the mean absorbance values (A) for each set of reference standards, controls, samples and blanks.
2. Subtract the value for blanks from those for standards, control and unknown samples.
3. Calculate the B/B)% values by dividing each value by the value for the zero-standard.
4. For the standards, plot a graph on semi-log graph paper with B/BO% values on the ordinate and the E2 concentrations (pg/mL) on the abscissa.
5. Using the graph read off the E2 concentrations for the unknown samples.
6. The values above the readable and below the readable range should be repeated using appropriate dilution.

### SENSITIVITY & EXPECTED VALUES

The sensitivity of the assay is 10pg/mL and each clinical laboratory should establish its own normal range based on the patient population. The results of randomly

### QUALITY CONTROL

Good Laboratory practice requires that quality control specimens be run with each standard curve to establish assay performance characteristics such as recovery, linearity, precision and specificity. The average recovery in this assay is in the range of 99.6% The recovery in the linearity range is about 98.5% and the linear range of the assay is 0-1000pg/mL. The intra-assay variation 10.5% and inter assay variation is about 8.5% The specificity was assessed by determining the crossreactivity of several known steroids in the assay and found less than 0.4% with estrone and 0.24% with estriol but others showed no significant crossreactivity.

### LIMITATIONS OF THE TEST

1. The E2 ELISA system designed here is for estimation of E2 levels in Primate samples by a professional only.
2. The wells should be adequately washed to obtain reproducible results. The washing step is extremely important and should be followed according to the instructions.
3. The results obtained with this assay should only be used as an adjunct to other diagnostic procedures and information available to the physician.
4. The assay should be performed by trained and skilled professional only.

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### Primate ESTRADIOL ELISA Test Kit

### Product Profile and Instructions

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