ESTRADIOL

BOVINE ESTRADIOL ELISA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE

The Microwell Estradiol ELISA TEST is an enzyme immunoassay system for quantitative determination of 17 beta Estradiol levels in bovine and related species serum. The test is intended for professional use as research tool in monitoring of conditions related to serum/plasma levels of Estradiol. The test kit has been designed to be used by a trained, skilled laboratory professional only.

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 of E2 antibody on a coated 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 450 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

Materials Provided

1. Microtiter wells 96, coated with second antibody.
2. Enzyme Conjugate solution, 12mL.
3. TMB Color Reagent, 12 mL
4. Stopping Solution (2N HCL), 6mL
5. 20 X Wash Buffer, 20 mL.
6. Sample diluent, 20mL
7. E2 Standard Set: 0, 10, 25, 100, 500 and 2500, 10,000 pg/ml/ 0.5ml/Vial
8. Quality control set QC1, (~100pg/mL) and QC2, (~500 pg/mL)
9. 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 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 lab, 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 (NaN3) 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 4-8 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 4-8 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 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 4-8 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.

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 50 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. And incubate at 37ºC 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 microwell 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.

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 5pg/mL and each laboratory should establish its own normal range based on the number of samples and for each species. A 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%

Specificity: The specificity was assessed by determining the crossreactivity of several known steroids (at 100ng/mL) in the assay and found no reactivity.

Limitations & Warranty

The present ELISA is designed for helping the scientist to analyze test samples only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the ELISA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

LIMITATIONS OF THE TEST

1. The E2 ELISA system designed here is for estimation of E2 levels in rodent samples by a laboratory 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 assay should be analyzed under GLP and GMP conditions where ever applicable.

BIBLIOGRAPHY

1. Webster J., 1987 understanding the dairy cow. BSP professional Books. Oxford p64-68, 300-321, and 343-344.
2. Robinson T.J. 1977 Reproduction in cattle "In reproduction in Domestic animals" 3rd ed.HH.Cole & PT Cupps editors, p439-442
3. Bulman DC.& Lamming GE. 1978 Milk progesterone levels interrelation to conception, repeat breeding and factors influencing acyclicity in dairy cows. J Reprod Fertil.54, 447-458
4. Ruiz FJ et al. 1992 Cost benefit evaluation of on-farm milk progesterone testing to monitor return to cyclicity and to classify ovarian cysts. J Dairy Sci 1992 75(4), 1036-1043
5. Autrere MB and Benson H 1976 Progesterone: an overview and recent advances Jour Par Sci 65, (6)783-800
6. Chattoraj SC 1976 Endocrine function in Fundamentals of Clinical Chemistry, NW Tietz eds.,WB Saunders, Chap 13, 699-823