Bovine ESTRONE SULFATE

PRODUCT PROFILE AND OPERATING INSTRUCTIONS

INTENDED USE

The Microwell Estrone Sulfate (ES) Elisa is an enzyme immunoassay system for quantitative determination of Estrone Sulfates levels in serum/plasma/urine samples of Bovine and related species. The test is intended for professional use as research tool in monitoring of conditions related to serum/plasma/urine levels of Estrone Sulfates.

PRINCIPLES OF TEST

The ES 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 ES to be assayed (unlabeled antigen) is added to a standard amount of a conjugated ES (labeled antigen). The labeled and unlabeled antigens are then allowed to compete for high affinity binding sites of ES antibody on coated on to the plate for two hours. 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 6 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 ES in serum samples

MATERIALS PROVIDED	**Materials Required (Not Provided)**
1. Antibody-coated microtiter wells, 96-well plate	1. Semiautomatic pipettes: 20ul and 200ul
2. Reference Standard, 0.6ml (0, 1.0, 5.0, 10, 25, 50 ng/mL)	2. Disposable pipette tips
3. Enzyme Conjugate Reagent, 12 mL	3. Microtiter plate shaker
4. TMB Color Reagent, 12 mL	4. Microtiter well reader.
5. 20X Wash buffer, 20 mL	5. Plate washer
6. Stop solution (2N HCl), 6mL	6. Absorbent paper
7. Instructions	7. 37 C incubator
	8. Parafilm to cover plate
	9. Distilled water

PRECAUTIONS

The bovine Estrone sulfates (Total) ELISA test kit is a complete kit with all the required reagents, identify and mark them as soon as you open the kit.

1. This kit contains reagents manufactured from blood products and samples should be considere potentiallyinfectious 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 and 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.

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 manufacturer 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

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.
Dilute concentrated specimen samples with Standard/sample dilution buffer and mix well before use in the assay.

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 50 ul of standards and samples into appropriate wells.
   Add 100 ul of ES Enzyme Conjugate Solution to each well (except those set for blanks),
   Incubate at 37C for 2 hours.
   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 solution into each well (including the blanks). Remember the 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 ES concentrations (ng/mL) on the abscissa.
5. Using the graph read off the ES concentrations for the unknown samples.

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 0.5 ng/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 0-50 ng/mL. The intra-assay variation 10.5% and inter assay variation is about 8.5%

Specificity: The specificity was assessed by determining the cross-reactivity of several known steroids (at 200ng/mL) in the assay and found no reactivity,

LIMITATIONS OF THE TEST

The ES ELISA system designed here is for estimation of ES levels in bovine samples by a laboratory professional only.
The wells should be adequately washed to obtain reproducible results. The washing step is extremely important and should be followed according to the instructions.
The assay should be analyzed under GLP and GMP conditions wherever applicable.

REFERENCES

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Bovine ESTRONE SULFATE/ESTRONE GLUCURONIDE ELISA TEST KIT

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