

CANINE ESTRADIOL

CANINE ESTRADIOL ELISA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE

The Microwell Estradiol Elisa kit is an enzyme immunoassay system for quantitative determination of Estradiol levels in Canine 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 is designed for a trained, skilled laboratory professional use 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 limited number of secondary 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 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. Rabbit anti E2 antibody 7.0 mL
3. Enzyme Conjugate solution, 12 mL.
4. E2 Standard Set: 0,10, 30,100, 300, 1200, 5000pg/mL.
+ QC1 (range 50pg/ml) and QC2 (range 300 pg/ml)
5. TMB Color Reagent, 12 mL
6. Stopping Solution (2N HCL), 6 mL
7. 20 X Wash Buffer, 20 mL.
8. Instructions

Materials Required (Not Provided)

1. Semiautomatic pipettes: 20ul and 200ul
2. Disposable pipette tips
3. Microtiter plate shaker
4. Microtiter well reader.
5. Plate washer
6. Absorbent 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.
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 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.

ASSAY PROCEDURE

1. All reagents should be allowed to reach room temperature (18-25C) before use.
2. Pipette 25 ul of standards, samples, and controls into appropriate wells.
3. Add 100 ul of E2 Enzyme Conjugate Solution to each well (except those set for blanks),
4. Add 50 uL of anti rabbit E2 antibodies and 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 solution 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 OF THE TEST

1. The E2 ELISA system designed here is for estimation of E2 levels in Canine 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.

REFERENCES

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