CANINE ALDOSTERONE

CANINE ALDOSTERONE ELISA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE:

The Aldosterone ELISA test is an immunoassay designed for the quantitative determination of Aldosterone in serum/plasma. The test is intended for professional use as an aid in the diagnosis and monitoring of physiological/pathological conditions related to serum/plasma Aldosterone in Canine and related species.

PRINCIPLES OF TEST

The Aldosterone Quantitative Test is based on a widely used immunoassay technique. A sample (serum/ plasma) containing an unknown amount of Aldosterone to be assayed (unlabeled antigen) is added to a standard amount of a labeled derivative of the same substance (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. In this kit an enzyme label is used. The biospecfic reaction takes place during 2hour incubation. After washing away, substrate solution is added and the enzyme 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 Aldosterone in serum/plasma samples of K9 and related species. It may also be used following an extraction procedure for assaying urinary Aldosterone (call for details of the procedure). Note: The Aldosterone levels should be established in your laboratory using your own set of samples and standards and good laboratory practice should be employed where applicable.
NOTE: THIS IS SPECIFICALLY DESIGNED TO MEASURE ALDOSTERONE IN CANINE AND RELATED SPECIES.
ALL HUMAN ALDOSTERONE KITS AVAILABLE IN THE MARKET DO ADDRESS THE INTERFERENCE OF MATRIX EFFECT.
ENDOCRINE IS SPECIFICALLY DESIGNED TO REMOVE MATRIX EFFECT FROM SERUM OR PLASMA.

Materials Provided

1. Microtiter wells coated with Aldosterone specific antibody.
2. Enzyme labeled (HRP) Aldosterone solution, 12mL
3. Aldosterone Standards, 1 Set ready to use, 0.5ml/vial
   (Contains 0, 10, 50,200,1000,5000,10,000 pg/ml
4. TMB Color Reagent (One-step ready to use), 12 mL
5. Stop Solution (2N HCl), 6 mL
6. 20x Wash Buffer, 20 mL.
7. Sample diluent, 20mL
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. CAUTION: This kit contains reagents manufactured from animal serum/plasma components. The source materials have been tasted by immunoassay for hepatitis B surface antigen and found to be negative. Nevertheless, all 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 labs or local and state.
2. The contents of this kit, and their residues, must not come into contact with ruminating animals or swine.
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 CONDITIONS

1. Store the kit at 4-8 C upon receipt and when it is not in use.
2. Keep microtiter wells in a sealed bag with desiccants to minimize exposure to damp air.
3. After every use place the caps tightly.

INSTRUMENTATION

A microtiter well reader with bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 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 grossly hemolytic or lipemic samples should not be used may affect results. Samples with bilirubin may also interfere with the assay.
2. A venous blood sample (enough to produce about 0.5 ml serum) is collected aseptically.
3. Preparation of the samples: Mix 0.05mL of serum with 0.20mL of sample diluent and add 0.05mL per well. The samples diluted can be stored at –20C for further use. Dilution of sample will eliminate adding very low volume (10ul).

REAGENT PREPARATION

1. Allow all the kit contents to stand 30-60 minutes at room temperature before use.
2. Read the instructions well and under stand before starting the assay system.
3. All the test procedures must be carried-out from start to Finnish with out interruption.
Use disposable tips for each sample and do not mix.
4. Mix Wash buffer 1 part with 19 parts of distilled water.
5. Highly concentrated samples should be diluted wit sample diluent (eg. 1:5, or 1:10), to bring on to a readable range on the curve.

ASSAY PROCEDURE

1. Pipette 50ul of standards (ready to use do not dilute them)
3. Add 100ul of Aldosterone Enzyme Conjugate Solution to each well (except those set aside for blanks).
4. Incubate for 2 hours at 37C, temperature.
5. Terminate the reaction and wash the plate 4-5 times with Wash Solution (250-300ul) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture,
6. Add 100 ul of TMB color reagent into each well (including the blanks). Remember for pipetting order.
7. Incubate the plate for 20 minutes without shaking.
8. Stop reaction by adding 50ul of Stopping Solution (a drop) to each well in the same sequence that the Substrate Solution was added. Gently mix for 1-2 minutes.
9. Read the absorbency at 450nm with a microwell reader.

NOTE: The substrate incubation should be carried out at room temperature (within the temperature range 25-28C). For temperature outside this range, the duration of the incubation should be adjusted by approximately 1 minute/1C.

CALCULATION OF RESULTS

1. Calculate the mean absorbance values (A) for each set of reference standards, controls (set up your own internal 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 Aldosterone concentrations (ng/mL) on the abscissa.
5. Using the graph read off the Aldosterone concentrations for the unknown samples.
6. You may use any commercial assay soft-ware to analyze the data.

EXPECTED VALUES AND SENSITIVITY

It is recommended that each laboratory should establish values to reflect differences specific to experimental conditions. The minimum detectable concentration of Aldosterone by this assay is estimated to be 50 pg/ml.

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.

REFERENCES

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Quality Control Data:
It is highly recommended that each laboratory must establish their own internal controls and normal reference values for desired pharmacological and physiological parameters.

A typical standard curve (illustration only) for Canine Aldosterone is given below:

Standard pg/mL	OD at 450nm
10,000	0.34
5000	0.53
1000	0.76
200	1.11
50	1.74
10	2.25
0	2.59

ELISA Performance Characters

Precision: Inter and Intra assay variation (CV) was determined from several different pooled serum samples in three different experiments.

Inter-assay variation	Set1: CV= 5.8 % (N=10)	Set2: CV= 6.8 % (N=10)	Set3: CV= 7.2 % (N=10)
Intra-assay variation	Set1: CV= 6.2% (N=10)	Set2: C= 5.8 % (N=10)	Set3: CV= 8.1 % (N=10)

Sensitivity: The lowest level detectable in this assay is 50pg/mL of serum or plasma

Specificity: The Canine Aldosterone ELISA system utilizes highly specific antibody to coat on to the plate. The cross reactivity to other related hormones is not detectable under the sensitivity of the assay system.

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