CORTICOSTERONE

RODENT CORTICOSTERONE ELISA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE:

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

PRINCIPLES OF TEST

The Corticosterone Quantitative ELISA Test is based on a widely used immunoassay technique. A sample (serum/ plasma/urine) containing an unknown amount of Corticosterone 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 HRP enzyme label is used. The biospecific reaction takes place during 2 hour incubation at 37°C. 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.

Note: This kit is suitable for the direct measurement of Corticosterone in serum/plasma/urine samples of rodents only. The kits is designed and developed with Rodent serum to avoid matrix complications. Do not mix or use with other kits or components.

The Corticosterone levels should be established in your laboratory using your own set of samples and standards and good laboratory practice should be employed where applicable.

Materials Provided

1. Microtiter wells coated with Corticosterone specific antibody.
2. Enzyme labeled (HRP) Corticosterone solution, 12mL
3. QC Controls: Low (1-2 ng/ml) and high range (5-10).
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, 25 mL
8. Corticosterone, (Standards/Calibrators:)1 Set. (0, 0.5, 1, 2, 5, 10, 20 ng per mL). 0.5mL/Vial
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. CAUTION: This kit contains reagents manufactured from serum/plasma components. The source materials have been tasted by immunoassay for hepatitis B surface antigen and antibodies to HIV virus 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 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.(Call Endocrine for your ELISA reader requirements)

SPECIMEN COLLECTION AND PREPARATION

1.This kit is suitable for use with serum or heparin plasma samples. The use of grossly hemolytic or lipemic samples will 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.

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.
4. Use disposable tips for each sample and do not mix.
5. Mix Wash buffer 1 part with 19 parts of distilled water.
6. Highly concentrated samples should be diluted with Sample diluent provided.

ASSAY PROCEDURE

1. Pipette 50 ul of standards (ready to use and do not dilute)
2. Sample 50 ul samples into appropriate wells
3. Add 100ul of Corticosterone Enzyme Conjugate Solution to each well (except those set aside for blanks).
4. Incubate for 2 hours at 37C.
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 450 nm with a microwell reader.

NOTE: The substrate incubation should be carried out 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, 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 Corticosterone concentrations should be (ng/mL) on the abscissa.
5. Using the graph read off the Corticosterone concentrations for the unknown samples.
6. You may use any commercial assay software to analyze the data.

SENSITIVITY

It is recommended that each laboratory should establish values to reflect differences specific to experimental conditions. The minimum detectable concentration of Corticosterone by this assay is estimated to be 0.1 ng/ml.

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 rodent Corticosterone is given below:

Standard ng/mL	**OD at 450nm**
20	**0.28**
10	**0.43**
5	**0.59**
2	**0.98**
1	**1.34**
0.5	**1.98**
0	**2.43**

ELISA Performance Characters

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

Inter-assay variation	Set1: CV= 5.9 % (N=10)	Set2: CV= 6.3 % (N=10)	Set3: CV= 6.1 % (N=10)
Intra-assay variation	Set1: CV= 6.6% (N=10)	Set2: CV= 5.9 % (N=10)	Set3: CV= 5.2 % (N=10)

Sensitivity: The lowest level detectable in this assay is 0.1 ng/mL of serum or plasma

Specificity: The rodent Corticosterone 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.