RODENT ACTH

RODENT ADRINOCORTICOTROPIC HORMONE (ACTH) ELISA TEST KIT

Lyophilized standards

INTENDED USE

RODENT ACTH ELISA is intended to quantitative determination of Rodent Adrenocorticotropic hormone (ACTH) concentration in serum/plasma of Rodent and related species. The test is designed as research tool in evaluation of R &D, preclinical and clinical samples in Rodent and related species and should be employed by a trained/skilled professional.

INTRODUCTION

ACTH is Peptide hormone (is also called corticotrophin) is secreted by the anterior pituitary gland and is under the influence of hypothalamic Corticotrophin Releasing Factor (CRF). It has 36 amino acids in length. There are a lot of structural similarities of ACTH between species and is known as a stress hormone. For example, ACTH promotes steroid genesis in kidney. Many factors are known to influence the rate of ACTH secretion, including periods of sleep and wakefulness, exercise, stress, hypoglycemia and corticosteroids.

The Rodent ACTH Enzyme Immunoassay provides a rapid, sensitive and reliable result in 3 hours.

TEST PRINCIPLE

The rACTH Quantitative ELISA Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes highly specific anti-rACTH specific antibody for solid phase (microtiter wells) immobilization and ACTH-monoclonal antibody enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies on the plate and HRP Conjugated ACTH molecules creating specific sandwich of the antigen between two different epitopes of antibodies.. After 3 hours of incubation at 37ºC, the wells are washed with water to remove unbound-ACTH HRP. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of rACTH is directly proportional to the color intensity of the test sample.

MATERIALS PROVIDED

1. Antibody-coated microtiter wells, 96-well plate
2. Enzyme -Conjugate reagent 3mL
3. Lyophilized Reference Standards (0, 0.05, 0.25, 1.5, 5.0, 10ng/ml) Reconstitute in 1.5ml using standard/sample Diluent.
4. TMB Color Reagent, 12mL
5. Stop solution (2N HCl), 6mL
6. 20x Washing Buffer, 20mL.
7. Sample/Standard diluent, 20ml
8. Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Distilled water
4. Glass tubes or flasks to prepare TMB Solution
5. Vortex mixer or equivalent
6. Absorbent paper
7. Graph paper
8. Microtiter plate reader

SPECIMEN COLLECTION AND PREPARATION

Serum/plasma should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum or plasma samples only and not for whole blood. The Rodent test samples (plasma or serum) should be collected fresh and repeated freeze and thaw should be avoided, if the test samples are not analyzed immediately should be stored at -20C in small aliquots and take one aliquot at a time for analysis.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 4-8^{O}C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25^{O}C) before use.
2. All lyophilized standards provided with the kit should be diluted to 1.5ml using standard/sample diluent. This can be stored frozen at -20C for long term use.

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ASSAY PROCEDURE

One must follow accurately these steps to ensure correct results. Use clean pipettes and disposable tips

1. Secure desired number of coated wells in the holder.
2. Dispense 200ul of standards, specimens, and controls into appropriate wells
3. Add 25ul of ACTH-HRP conjugate and shake for 2-5 minutes on a shaker (about 300rpm) and incubate for 3 hours at 37C
4. Remove the incubation mixture by dumping plate contents into a waste container.
5. Rinse and dump the microtiter wells five (5) times (300ul) with dilute wash buffer.
6. Dispense 100 ul of TMB solution into each well. Gently mix for 10 seconds.
7. Incubate at room temperature for 20 minutes in the dark.
8. Stop reaction by adding 50 ul of stop solution (2N HCl) to each well.
9. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
10. Read optical density at 450nm with a microtiter well reader.

Important note: The wash steps are very critical and insufficient washing will result in poor precision and falsely elevated absorbency readings.

CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and test samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph, with absorbency values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of rACTH ng/mL from the standard curve.

EXPECTED VALUES AND SENSITIVITY

It is recommended to establish your local laboratory conditions for normal range in your laboratory animals.
Minimum detectable levels in this assay will be 50pg/ml

LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedures are carried out with understanding of the package insert instructions and adherence to good laboratory practice.
The wash step is extremely important and should follow for clean background and good reproducible results. Incubation conditions should be carefully monitored or establishing conditions at 37C should make adjustments for consistent and reproducible results.
Note: The components of this kit should not be mixed are used with other manufacturer kits.

LIMITATIONS & WARRENTY

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

1. Fisher DA 1977 Evaluation of anterior pituitary function In Radioimmunoassay Manual Eds. Nicholas AL and Nelson JCP 3498 Nichols Institute.
2. Reichlin S et al. 1976 Hypothalamic hormones Ann Rev Med 27p359

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