Primate CORTISOL

PRIMATE CORTISOL ELISA TEST KIT

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE:

The Cortisol ELISA test is an immunoassay designed for the quantitative determination of cortisol in serum/plasma/urine. The test is intended for professional use as an aid in research, development and monitoring of physiological/pathological conditions related to serum/plasma/urine cortisol in Primates (monkeys) and related species.

PRINCIPLES OF TEST

The Cortisol Quantitative Test is based on a widely used immunoassay technique. A sample (serum/ plasma/urine) containing an unknown amount of cortisol 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 1 hour 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 cortisol in serum/plasma/urine samples. It may also be used following an extraction procedure for assaying urinary cortisol (call for details of the procedure). Note: The cortisol 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 cortisol specific antibody.
2. Enzyme labeled (HRP) Cortisol solution, 12mL.
3. TMB Color Reagent (One-step ready to use), 12 mL
4. Stop Solution (2N HCl), 6 mL
5. 20x Wash Buffer, 25 mL.
6. Sample diluent, 25mL
7. Cortisol Standards, 1 Set. (Contains 0, 5, 10,30,100,300,600 ng/mL).
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 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 OF TEST KIT AND INSTRUMENTATION

Note of Caution: Immediately after receiving the kit all standards, if not used, should be kept at -20^{O}C. Unopened test kits should be stored at 2-8^{O}C. The microtiter plate should always be kept in a sealed bag with desiccants to minimize exposure to damp air at room temperature. 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, with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

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 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.
3. If the expected concentrations are higher in the linear range, you should dilute the test sample (eg:1;5 ect.) and add the ELISA.

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 wit sample diluent (eg. 1:5, or 1:10), to bring on to a readable range on the curve.

ASSAY PROCEDURE

1. Pipette 25ul of standards (ready to use and do not dilute)
2. Test sample 25ul into appropriate wells.
3. Add 100ul of Cortisol Enzyme Conjugate Solution to each well (except those set aside for blanks).
4. Incubate for 1 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 Cortisol concentrations (ng/mL) on the abscissa.
5. Using the graph read off the Cortisol 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 cortisol by this assay is estimated to be 1 ng/ml.

REFERENCES

1. BONDY PK. The adrenal cortex, in Randy PT, Rosenberg LE., Metabolic control and disease (8 ed) 1980, WB Sanders, Philadelphia, p1427-1499.
2. Lambert A Clinical Endocrinology 1974, Springer-Verlag New York, p299-305
3 Veosei P, Glucocorticoids, Cortisol, corticosterone, Compound S in Jaffe BM, Behrman HR 1974 in Methods in Radioimmuno assay p393-411.
3. Spark R 1971, Simplified assessment of pituitary-adrenal reserve Annals of internal Med. 75 p75-717
4. Regestein QR, et al 1986 Behav Neural Biol, Vol 45(3)p329-341
5. David M. Lyons et al 2000, Journal of Neuroscience, Vol 20(20)p7816-7821

Revised 052008-1

35325 Fircrest Street, Newark, CA 94560-1003 * Phone (800) 745-0843 * (510) 745-0844 * Fax (510) 745-0977
www.endocrinetech.com