Cortisol Test System
Product Code: 3625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Cortisol Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cortisol (hydrocortisone, compound F) is the most potent glucocorticoid produced by the human adrenal cortex. As with other adrenal steroids, cortisol is synthesized from cholesterol, through a series of enzymatically mediated steps, by the adrenal cortex.1-3 The first and rate-limiting step in adrenal steroidsogenesis, conversion of cholesterol to pregnenolone, is stimulated by plurihormonal adrenocorticotropic hormone (ACTH) which is, in turn, regulated by hypothalamic corticotropin releasing factor (CRF). ACTH and CRF synthesis are influenced by a variety of factors. In plasma, the major portion of cortisol is bound with high affinity to corticosteroid-binding globulin (CBG), although it remains loosely bound to albumin. Physiologically effective in anti-inflammatory activity and blood pressure maintenance, cortisol is also involved in gluconeogenesis, protein synthesis, intracellular receptors and has effects in numerous other physiologic systems, including immune function, glucose-counter regulation, vascular tone, substrate utilization and intermediary metabolism.3-4 Cortisol is excreted primarily in urine in an unbound (free) form.

Cortisol production has an ACTH-dependent circadian rhythm with peaks in the early morning and a nadir at night. The factors controlling the excretion of cortisol are not completely defined. The circadian rhythm of ACTH/cortisol secretion matures gradually during early infancy, and is disrupted in a number of physical and psychological conditions.5 Furthermore, increased amounts of ACTH and cortisol are secreted independently of the circadian rhythm in response to physical and psychological stress.6

Elevated cortisol levels and lack of diurnal variation have been identified in patients with Cushings disease (ACTH hyper secretion).7 Furthermore, circadian cortisol levels have also been identified in patients with adrenal tumors. Low cortisol levels are found in primary adrenal insufficiency (e.g. adrenal hypoplasia, congenital adrenal hyperplasia, Addison's disease) and in ACTH deficiency.8-10 Due to the normal circadian variation of cortisol levels, distinguishing normal and abnormally low cortisol levels can be difficult. Therefore, various tests to evaluate the pituitary-adrenal (ACTH/cortisol) axis function are available. A 24-hour urine-free cortisol, short- and long-term ACTH stimulation, CRF stimulation and artificial blockage of cortisol synthesis with metronemone have been performed.11

Cortisol response characteristics for each of these procedures have been reported. The Monobind Cortisol EIA Kit uses a specific monoclonal anti-cortisol antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally-occurring steroids is low.

The employment of several serum reference values of known cortisol concentration permits construction of a graph of activity and concentration. From comparing the data to the response curve, an unknown specimen's activity can be correlated with cortisol concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay are antibody, antigen, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results. Between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:

$$\text{Ag} + \text{Ab} \rightleftharpoons \text{AgAb}$$

$$k_a = \text{Rate Constant of Association}$$

$$k_d = \text{Rate Constant of Disassociation}$$

$$K = \frac{k_a}{k_d}$$

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction from degradation or aspiration.

$$\text{AgAb} + \text{AbBtn} \rightleftharpoons \text{AgAbBtn}$$

The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum concentrations of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Cortisol Calibrators – 1mLvial – Controls A-F
Six (6) vials of serum reference for Cortisol at concentrations of 0 (A), 1.0 (B), 4.0 (C), 10.0 (D), 20.0 (E) and 50.0 (F) µg/dl. Store at 2-8°C. A preservative has been added.

B. Cortisol Enzyme Reagent – 7.0 ml/vial – Icon
One (1) reconstituted (or in original vial) (Analog) – horseradish peroxidase (HRP) conjugate in a protein stabilizing matrix with buffer, red dye, preservative and binding protein as inhibitors.

C. Cortisol Biotin Reagent – 7.0 ml – Icon
One (1) vial containing anti-cortisol biotinated mlgG conjugate in buffer and preservative.

D. Streptavidin Coated Plate – 96 wells – Icon
One (1) 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon
One (1) vial containing a conjugate on buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial – Icon
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial – Icon
One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

H. Stop Solution – 1ml/vial – Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for (60) days when stored at 2-8°C.

II. PROCEDURE

4.1 Required But Not Provided:

A. Apparatus:

1. Computer data reduction software designed for ELISA
2. 96-well microplate reader capable of absorbance capability.
3. Wash Buffer
4. Wash Solution
5. 0.100ml working substrate solution
6. 0.050ml stop solution
7. Timer.

B. Reagents:

1. A ready to use vial containing Cortisol (Analog) - conjugate for a limited number of antibody binding sites. The reagents are stable for sixty (60) days when stored at 2 - 8°C.
2. Plastic wrap or microplate covers for incubation steps.
3. Wash Buffer (prepared on site with distilled or deionized water).

4. To determine the concentration of cortisol for an unknown, a dose response curve is needed.

5. A dose response curve is used to ascertain the concentration of cortisol.

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10. A dose response curve is used to ascertain the concentration of cortisol.

11. To provide a reference wavelength of 620-630nm to minimize well imperfections in a microplate reader. The results should be read within thirty (30) minutes of adding the substrate.

Note: Dilute the samples suspected of concentrations higher than 50 µg/dl and 1:10 with cortisol 0 µg/dl patient serum.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of cortisol.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Place a 0.100µl working substrate solution reference versus the corresponding cortisol concentration in µg/dl on a linear graph paper (do not average the duplicates of the serum reference values prior to this step).
3. Connect the points with a best-fit curve.
4. The horizontal axis of the graph (the duplicates of the unknown on the vertical axis of the graph, find the intersecting point between the µg/dl and the horizontal axis). The result is the concentration of cortisol.

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and gather device usage.

11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

12. Risk Analysis- as required by CE Mark IV Directive 93/42/EEC for this and other devices, made by Monobind, can be obtained via email from Monobind@monobind.com.

12.1 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

3. If more than one plate is used, it is recommended to repeat the sample(s) which is terminated by the addition of the stop solution.

4. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution.

5. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.

6. Four out of six quality control pools should be within the established range.

7. It is important that the time of reaction in each well is held constant to achieve reproducible results.

8. Use components from the same lot. No intermixing of reagents from different batches.

9. Use components from the same sequence to eliminate any time-deviation during reaction.

10. If test kits are altered, such as by mixing parts of different kits, the sensitivity of the method, the population tested and factors: the specificity of the method, the population tested and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

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13. A study of normal adult population was undertaken to determine expected values for the Cortisol AccuBind® ELISA Test System. The mean (R) values, standard deviations (σ) and expected ranges (±2σ) are presented in Table 1.

14. Only slight amounts of bias between this method and the reference method is indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

15. The Cortisol AccuBind® ELISA Test System was compared with a coated tube radioimmunoassay method. Biological specimens from low, normal and high cortisol level populations were used. The values ranged from 0.4 µg/dl – 95 µg/dl. The total number of such specimens was 202. The least square regression equation and correlation coefficient were computed for this cortisol ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

11.0 QC. PARAMETERS

1. The absorbance (OD) of calibrator 0 µg/dl should be > 1.3.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

3. The reagents for the procedure have been formulated to avoid erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoadassays. (Boscato LM Stuart MC.'Heterophilic antibodies; a problem for all immunoadassays. J of Steroid Biochemistry, 7, 785-791 (1975).

12.0 RISK ANALYSIS

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

3. If more than one plate is used, it is recommended to repeat the sample(s) which is terminated by the addition of the stop solution.

4. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution.

5. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.

6. Plate readers measure vertically. Do not touch the bottom of the wells.

7. Failure to remove the substrate solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

8. Use components from the same lot. No intermixing of reagents from different batches.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential.