3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

The essential reagents required for a enzyme immunoassay (EIAs) include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigenic, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The reaction is illustrated by the following equation:

\[ E + A + AgAbBtn \rightarrow EnzAgAbBtn + AgAbBtn \]

where:
- \( E \) = Enzyme-antigen Conjugate (Constant Quantity)
- \( A \) = Native Antigen (Variable Quantity)
- \( AgAbBtn \) = Antigen-Antibody Complex
- \( EnzAgAbBtn \) = Antigen-Enzyme Conjugate - Antibody Complex

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 after decantation or aspiration. The equation is simplified to:

\[ K = k_a / k_d = \text{Equilibrium Constant} \]

The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different dilutions of the calibration standard, a best-fit curve is generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:
- A. Calibrators – 1ml/vial - Icons A-F
- B. Cortisol Enzyme Reagent – 1.0 ml/vial - Icon
- C. Steroid Conjugate Buffer – 7.0 ml/vial - Icon
- D. Cortisol Biotin Reagent – 7.0 ml/ml - Icon
- E. Substrate A – 7ml/vial - Icon
- F. Wash Concentrate Solution – 2ml - Icon
- G. Substrate B – 7ml/vial - Icon
- H. Cortisol Calibrators – 1ml/vial - Icons A-F
- I. Cortisol Biotin Reagent – 1.0 µl/vial - Icons A-F
- J. Stop Solution – 8ml/vial - Icon

Each laboratory should assay controls at levels in the low, normal and abnormally low cortisol levels can be identified with ease. Therefore, various tests to evaluate the pituitary-adrenal (ACTH-cortisol) axis, such as insulin-induced hypoglycemia, short- and long-term ACTH stimulation, CRF stimulation and adrenal blockade cortisol synthesis with metyrosine have been performed [8-13]. Cortisol concentration characteristics for each of these procedures have been reported.

Monobind Cortisol ELISA 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 references of known cortisol concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with cortisol concentration.

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 [reviewed in 1, 2, and 9]. 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, such as insulin-induced hypoglycemia, short- and long-term ACTH stimulation, CRF stimulation and adrenal blockade cortisol synthesis with metyrosine have been performed [8-13]. Cortisol concentration characteristics for each of these procedures have been reported.

Monobind Cortisol ELISA 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 references of known cortisol concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with cortisol concentration.

10.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (O.D.) of calibrator 0 µg/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

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

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Cortisol AccuBind™ Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**TABLE 2**

<table>
<thead>
<tr>
<th>Sample N</th>
<th>X</th>
<th>σ</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>16</td>
<td>14.2</td>
<td>0.91</td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
<td>14.2</td>
<td>0.91</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>36.5</td>
<td>2.23</td>
</tr>
</tbody>
</table>

Note: The data presented in Example 1 and Figure 1 is for illustration purposes only and should not be used in lieu of a standard curve prepared with each assay.

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