Dehydroepiandrosterone (DHEA) Test System
Product Code: 7425-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Dehydroepiandrosterone (DHEA) is a C19 steroid secreted by the adrenal cortex, and is a precursor in testosterone and estrogen biosynthesis. Due to the presence of a 17-oxo (rather than hydroxyl) group, DHEA possesses relatively weak androgenic activity, which has been estimated at ~10% of that of testosterone.1

The physiologic role of DHEA is not well-defined. Since DHEA has a relatively low affinity constant for sex hormone binding globulin (SHBG), the bioactivity at the cell surface is maybe more significant than other androgenic steroids that have much higher affinity to SHBG. Abnormal levels have been reported in obesity and schizophrenia. Excessive DHEA secretion can cause hirsutism and virilization. DHEA measurement is important in the investigation of adrenal androgen production for adrenal hyperplasia and tumors. DHEA has a fast clearance turnover rate compared to its sulfated conjugate (Monobind Product 5125-300 DHEA-S). This leads to marked difference in circulation concentration compared to the sulfated derivative, which has much longer half life.2 DHEA levels do show circadian rhythm that reflects the secretion of ACTH and also varies during the menstrual cycle.

Measurement of serum DHEA is a useful marker of adrenal androgen synthesis. Abnormally low levels have been reported in hypoadrenalism,1 while elevated levels occur in several conditions; including virilizing adrenal adenoma and carcinoma,2-3 hydrocortisone deficiencies14 and some cases of female hirsutism.2 Since very little DHEA is produced by the gonads,1 measurement of DHEA may aid in the localization of the androgen source in virilizing conditions. The DHEA kit uses a specific anti-DHEA 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 DHEA 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 DHEA concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the antigen, antigens and antibody 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:

\[ e^{\text{Ag}} + g + Ag_{\text{AbBtn}} \rightarrow Ag_{\text{AbBtn}} + e^{\text{Ag} + g} \]

\[ Ag_{\text{AbBtn}} = \text{Biotinylated x-DHEA IgG Antibody (Constant Quantity)} \]

\[ Ag = \text{Native Antigen (Variable Quantity)} \]

\[ Ag_{\text{Antigen-Enzyme}} + e^{\text{Ag} + g} \rightarrow \text{immobilized complex} \]

\[ k_a = \text{Rate Constant of Association} \]

\[ k_r = \text{Rate Constant of Dissociation} \]

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 bond fraction after decantation asparagine. Immunoasay.

The enzyme activity in the antibody bond fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references 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. DHEA Calibrators – 1ml/vial - Icons A-F

B. DHEA Enzyme Reagent – 6ml/vial – Icon C

C. DHEA Biotin Reagent – 6ml/vial - Icon D

Dilute contents of wash solution to 1000ml with distilled or deionized water, pH 7.0 to 7.4. Store at 2-8°C.

Wash Buffer

Can be stored at room temperature (2-30°C) for up to 6 months.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and special handling and disposal procedures are required. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1989, HHS Publication No. (CDC) 88-8385.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or heparinized plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normative values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop veni-puncture tube with or without additives or anticoagulants (for serum) or evacuated tubes (containing EDTA or heparin) for plasma. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, low and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained for each lot. The patient sample(s) should set acceptable performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate uncontrolled change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.

Note 1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have variations.

2. Pipette 0.025ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.05ml (50µl) of the DHEA Enzyme Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix.

5. Add 0.05mol/l (50µl) of Anti-DHEA Biotin Reagent to all wells.

6. Close and microplate gently for 20-30 seconds to mix.

7. Cover and incubate for 60 minutes at room temperature.

8. Discard contents of wells by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant and blot or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash.

10. If the peak area of a control is outside the range of ±10% of the mean peak area of the controls, the results should be redone after reprocessing the specimen.

13. Read the absorbance in each well at 450nm using a reference wavelength of 620-630nm. The results should be read at 396-398 minutes of incubating the stop solution.

Note: Dilute the samples suspected of concentrations higher than 30ng/ml 1:5 with DHEA ‘0’ ng/ml calibrator.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of DHEA in unknowns. For In Vitro Diagnostic Use as outlined in Example 1.

Record the absorbance obtained from the printout of the microplate reader as outlined Example 1.

Plot the absorbance for each duplicate serum reference versus the corresponding DHEA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

Connect the points with a best-fit curve. For In Vitro Diagnostic Use as outlined Example 1.

To determine the concentration of DHEA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point with the best-fit curve, and read the unknown concentration in ng/ml on the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance of 0.8500 (ng/ml) was determined by placing a known on the horizontal axis, finding the best-fit curve, and reading the unknown concentration in ng/ml on the vertical axis of the graph.

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.
The addition of substrate solution initiates a kinetic reaction, on request from Monobind Inc., criteria should be met:

2. Pipetting of samples should not extend beyond ten (10)

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper 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 IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com

12.2 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. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophile antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. Heterophile antibodies: a problem for all immunoassays’ Clin. Chem. 1968:3427-33.) For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. If test kits are altered, such as by mixing parts of different kits, replication and spurious results.

6. It is important to keep in mind that establishment of a range of factors: the specificity of the method, the population tested and reasons, each laboratory should depend upon the range of values of hirsutism in pubertal and postpubertal women”, J Clin Endocrinol Metab, 73:674-686 (1991).

15.0 REFERENCES


14.3 Specificity

The % cross-reactivity of the DHEA antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of DHEA needed to displace the same amount of labeled analog.

Even though the absorbance (OD) of calibrator 0 ug/ml should be > 1.3, the sensitivity was ascertained by determining the variability of the 0 ug/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.2 Sensitivity

The DHEA AccuBind® ELISA Test System has a sensitivity of 0.10 ng/ml. The sensitivity was determined by testing the DHEA AccuBind® ELISA 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.

11.2 Risk Analysis

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

12.1 Assay Performance

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

Weighing of samples should not extend beyond ten (10) minutes to avoid assay drift.

Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.

If more than one (1) plate is used, it is recommended to repeat the dose response curve.

5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deprivation during reaction.

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

7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor reproducibility 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. Any deviation from Monobind’s IFU may yield inaccurate results.

14.0 Performance Characteristics

1. Precision

The within and between assay precision of the DHEA AccuBind® ELISA Test System were determined by analyses on three replicates of each sample.

The within assay precision (CV) was calculated as 95% confidence interval.

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

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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 test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophile antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. Heterophile antibodies: a problem for all immunoassays’ Clin. Chem. 1968:3427-33.) For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

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Revision: 2 Date: 2013-AUG-08 DCO: 0895 Product Code: 7425-300

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