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% that of testosterone.1

The physiologic role of DHEA is not well-defined. Since DHEA has 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.

Materials Provided:
A. DHEA Calibrators – 1ml/vial - Icons A-F
B. DHEA Enzyme Reagent – 6ml/vial - Icons
C. DHEA Biotin Reagent – 6ml/vial - Icons
D. Enzyme-antigen conjugate (Monobind Product 5125-300 DHEA-S).
E. Streptavidin CW
F. Substrate Solution – 12ml/vial - Icon SN
G. Stop Solution – 8ml/vial - Icon
H. Product Instructions.

DHEA has a fast clearance turnover rate compared to its sulfated conjugate (Mondirol Product 5125-300 DHEA-S). This leads to marked differences in elimination concentration compared to its sulfated derivative, which has much longer half life.2 DHEA levels do show circadian rhythm, as well as a negative correlation with the secretion of ACTH and also varies during the menstrual cycle.

Measurement of serum DHEA is a useful marker of adrenal hyperplasia and tumors.

3.0 PRINCIPLE

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

K = ka / k-a = Equilibrium Constant

AgAbBtn + EnzAgAbBtn + StreptavidinCW

AgAbBtn = Biotinylated x-DHEA IgG Antibody (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

AbBtn = Biotinylated x-DHEA IgG Antibody (Constant Quantity)

EnzAgAbBtn = Enzyme-antigen conjugate - Antibody Complex

Ka = Rate Constant of Association

k-a = Rate Constant of Dissociation

K = Kα/ka = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This results in the separation of the antibody bound fraction after decantation or aspiration.

The enzyme activity in the antibody bound 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.

3.1 Required But Not Provided:
A. DHEA Calibrators – 1ml/vial - Icons A-F
B. DHEA Enzyme Reagent – 6ml/vial - Icons
C. DHEA Biotin Reagent – 6ml/vial - Icons
D. Enzyme-antigen conjugate (Monobind Product 5125-300 DHEA-S).
E. Streptavidin CW
F. Substrate Solution – 12ml/vial - Icon SN
G. Stop Solution – 8ml/vial - Icon
H. Product Instructions.

4.0 REAGENTS

4.1 Required But Not Provided:
A. DHEA Calibrators – 1ml/vial - Icons A-F
B. DHEA Enzyme Reagent – 6ml/vial - Icons
C. DHEA Biotin Reagent – 6ml/vial - Icons
D. Enzyme-antigen conjugate (Monobind Product 5125-300 DHEA-S).
E. Streptavidin CW
F. Substrate Solution – 12ml/vial - Icon SN
G. Stop Solution – 8ml/vial - Icon
H. Product Instructions.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum sample versus the corresponding DHEA concentration in ng/mL on linear graph paper. For each serum sample, do not average the duplicates of the serum references before plotting.
3. Connect the points with a best-fit curve.
4. For use of for use 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 on the curve, and read the concentration (in ng/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance in the patient sample (1.68) intersects the corresponding DHEA concentration in ng/mL on the standard curve at 0.0505 ng/mL.

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.

8.0 REAGENT PREPARATION

8.1 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 (2-20°C) for up to 60 days.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators, and controls to room temperature (20 - 27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates’ wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Place and rinse microwell strips into the aluminum bag, seal, and store at 2-8°C.
2. Pipette 0.025ml (25µl) of the appropriate serum reference, control or patient specimen into each of the assay 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 0.050ml (50µl) of Anti-DHEA Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Replace and rinse strips at room temperature.
8. Discard the contents of the microplate 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 (tap 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. Add 0.100ml (100µl) of substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
11. Incubate at room temperature for twenty (20) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). The results should be read within five (5) minutes of adding 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 unknown samples.

Note: Software is utilized, the validation of the software should be ascertained.
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. Q.C. PARAMETERS

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

12. RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are 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.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. 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-deviation 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 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. Any deviation from Monobind’s IFU may yield inacurate results.

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.
3. The reagents for the test system procedure have been formulated to eliminate maximum 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 immunoassays. (Boscosa LM, Stuart MC. Heterophile antibodies: a problem for all immunoassays. Clin Chem. 1988;34:27-33).
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 mixing of different kits, which could produce false test results, or if results are incompletely interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Clinically, a DHEA-S value alone is not of diagnostic value and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.
8. The expected ranges for the DHEA AccuBind® ELISA Test System are detailed in Table 1.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a “normal” adult population, the expected ranges for the DHEA AccuBind® ELISA Test System are detailed in Table 1.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision
The within and between assay precision of 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.

15.0 REFERENCES

Revision: 3 Date: 2019-Jul-16 DCO: 1353
MP71425 Product Code: 7425-300