Dehydroepiandrosterone Sulfate (DHEA-S) Test System
Product Code: 5175-300

1.0 INTRODUCTION

The Quantitative Determination of Dehydroepiandrosterone Sulfate (DHEA-S) in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Dehydroepiandrosterone sulfate (DHEA-S) is the major C19 steroid secreted by the adrenal cortex, and is a precursor in the biosynthesis of testosterone. However, the bioactivity of DHEA-S may be due to the slower metabolic rate of the hormone in comparison to the rate of DHEA-S concentration. This may be due to the slower metabolic rate of testosterone. DHEA-S possesses relatively weak androgenic activity, which for this reason is often measured in female samples. 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 the antibody concentration of an unknown antigen.

4.0 REAGENTS

A. DHEA-S Calibrators – 6 ml/vial - ICON
Six (6) vials of serum reference for DHEA-S at concentrations of 0 (A), 0.2 (B), 1.0 (C), 5.0 (D), 4.0 (E) and 8.0 (F) in µg/mL. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by using 2.71 as the conversion.

For example: 1.0 µg/L x 2.71 = 2.71 µM/L

B. DHEA-S Tracer Reagent – 6.0 ml/vial – ICON
One (1) vial contains DHEA-S (analog)-horseradish peroxidase (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

C. DHEA-S Biotin Reagent – 6.0 ml/vial
One (1) bottle of reagent contains anti-DHEA-S biotinylated rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C.

D. Light Reaction Wells – 96 wells – ICON
96-well black plate. Store at 2-8°C.

E. Wash Solution – 20 ml/vial – ICON
One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Signal Reagent A – 7.0 ml/vial – ICON
One (1) vial contains hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

G. Signal Reagent B – 7.0 ml/vial – ICON
One (1) vial contains luminol in a buffer. Store at 2-8°C.

H. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents and controls should be stored at 2-30°C. Wash kit and control reagents are stable for 5 days. If the specimen(s) cannot be assayed within this period, the samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the samples are stored for more than 5 days, they should not be assayed. Store at 2-8°C.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of progesterone in unknown samples. 1. Record the RLUs obtained for the printout of the microplate reader as outlined in Example 1.

2. Plot the RLUs for each double serum reference versus the concentration of progesterone in ng/mL on linear graph paper.

3. Draw the best-fit curve through the plotted points. 4. To determine the concentration of DHEA-S for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the corresponding 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 by the average RLUs). If the average RLUs of the unknown intersects the calibration curve at 5.23 DHEA-S concentration (see Figure 1).

Note: Computer data reduction software designed for CLIA Accuracy may also be used for this calculation. If such software is utilized, the validation of the software should be ascertained.
11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following parameters must have been established.

1. The Dose Response Curve should be within established parameters.
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.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated sample(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 signal reagent initiates a kinetic reaction, therefore the signal reagent should be added in the correct sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermingling of reagents from different batches.
8. Patient specimens with DHEA-S concentrations above 6.0 ug/ml may be diluted (1:5, 1:10 or higher) with DHEA-S 0.01 N calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed is essential.
10. Any deviation from Monobind FL code 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 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 sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interactions between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays'. Clin Chem 1988:34-37). For diagnostic purposes, the results from this assay should be 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, as by mixing parts of different kits, which could produce false test results, or if results are incorrectly 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 calibrator 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.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the lower limit of the DHEA'S AccuLite® CLIA Test System are detailed in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mean (μg/ml)</th>
<th>Least Square Regression Coefficient</th>
<th>Correlation Coefficient</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S</td>
<td>0.0000</td>
<td>[y=0.1448+0.9858(x)]</td>
<td>0.982</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Only slight amounts of bias between this method 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.

**14.0 SPECIFICITY**

1. The cross reactivity of the DHEA-S antibody to selected substances was evaluated by adding interfering substances 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-S needed to displace the same amount of labeled analog.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dihydotestosterone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.0004</td>
</tr>
<tr>
<td>Dihydroepiandrosterone sulfate</td>
<td>0.0005</td>
</tr>
<tr>
<td>Estradiol</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**15.0 REFERENCES**