Dehydroepiandrosterone Sulfate (DHEA-S) Test System
Product Code: 5125-300

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 sulfate (DHEA-S) is the major C19 steroid secreted by the adrenal cortex, and is a precursor in testosterone and estrogen biosynthesis. DHEA-S, the sulfate ester of DHEA, is derived from sulfated precursors and by conversion of DHEA in adrenal and extracellular tissues. Due to the presence of a 17-oxo [rather than hydroxyl] group, DHEA-S possesses relatively weak androgenic activity, which for Due to the presence of a 17-oxo group, DHEA-S possesses relatively weak androgenic activity, which for its weak affinity for sex-hormone binding globulin.3

The physiologic role of DHEA-S is not well-defined. Serum levels are relatively stable throughout routine daily activities and conditions; including minimal adrenal androgen and carcinomas. 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies are rare causes of virilization.2 Since very high levels have been observed in 30% of patients with breast cancer, DHEA-S may play a role in breast cancer.2

3 Precipitation reaction and competitive reaction are used to estimate the concentration of antibodies bound to streptavidin immobilized on the well. 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 incubation at ambient temperature. 

A competitive reaction is illustrated by the following equation: 

\[
\text{AgAbBtn} + \text{EnzAgAbBtn} = \text{Streptavidin immobilized on well} 
\]

Note 1: Do not use reagents that are contaminated or have bacteria growth.

2.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal Use

Intended for 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 100% assurance that the test is appropriate for a specific agent or sample, all human serum samples should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biologicals in Microbiological and Immunological Laboratory," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8390.

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 normalcy, a fasting morning serum sample should be obtained. The blood should be collected in a red top veri-puncture tube with or without additives or anti-coagulants (for serum) or EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the plasma or serum from the cells.

In patients receiving therapy with high blood doses (i.e. nephrotoxic drugs), a fasting morning specimen (no less than 8 hours after the last biotin administration, preferably overnight to ensure fasting sample. Samples may be refrigerated at 2-8°C for a maximum period of 1-7 days, if fully refrigerated. A defrosted specimen should not be used. The sample should be placed in a refrigerated container for transport and stored at 2-8°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml (20µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every assay run.

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

8.0 REAGENT PREPARATION

1. Wash Buffer
2. Dilute contents of wash solution to 1000ml with deionized water in a suitable storage container. Diluted buffer
3. Store at 2-8°C. A preservative has been added. Store at 2-8°C. A preservative has been added. Store at 2-8°C.
4. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Follow (two 2 additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instructions for proper use. If a smooth bottle is employed, fill each well with the prescribed sample volume (100µl) as prescribed for the plate washer to dispose of the Was solution. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

Note 3: Avoid extended exposure to heat and light.

9. Add 0.100ml (100µl) of working substrate solution to all wells (see Section, 2.0 Considerations). Dilute contents of wash solution to 1000ml with deionized water in a suitable storage container. Diluted buffer
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

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

11. Inoculate at room temperature for fifteen (15) minutes. 12. Discard 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.

Note 3: Dispose of the reagents as per local guidelines.

12. Read the results in a spectrophotometer set at a reference wavelength of 260-630nm. The results should be read

Note 4: Dispose of the reagents as per local guidelines.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of DHEA-S in unknown specimens. 1. Record the absorbance obtained from the plot of the following reagent concentrations as indicated in Table 1. Calculate the concentration of an unknown sample using the following formula:

\[ \text{Concentration} = \frac{\text{A}_{\text{unknown}}}{\text{A}_{\text{standard}}} \times \text{Standard Concentration} \]

4. Plot the absorbance of each duplicate serum sample versus the corresponding DHEA-S concentration in ug/ml on linear graph paper. Include the results of each unknown sample on the graph. The data should be recorded in the following manner:

Note 5: Use a best-fit curve.

5. To determine the concentration of DHEA-S for an unknown, locate the average absorbance of the duplicates for each unknown sample. Plot the resulting average absorbance of each unknown sample on the dose response curve, and determine the concentration of DHEA-S from the curve.

Note 6: Use a best-fit curve.

6. Plot the absorbance of each duplicate serum sample versus the corresponding DHEA-S concentration in ug/ml on linear graph paper. Include the results of each unknown sample on the graph. The data should be recorded in the following manner:

Note 5: Use a best-fit curve.

7. The results should be read

Note 4: Dispose of the reagents as per local guidelines.

8.0 REAGENT PREPARATION

1. Wash Buffer
2. Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer
3. This may be due to the slower metabolic clearance rate of DHEA-S as compared to DHEA.

Note 3: Avoid extended exposure to heat and light.

4. Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels have been reported in hypoadrenalism,1 while elevated levels occur in several congenital adrenal glandular tumors. Since very high levels have been observed in 30% of patients with breast cancer, DHEA-S may play a role in breast cancer.2

The physiologic role of DHEA-S is not well-defined. Serum levels are relatively stable throughout routine daily activities and conditions; including minimal adrenal androgen and carcinomas. 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies are rare causes of virilization.2 Since very high levels have been observed in 30% of patients with breast cancer, DHEA-S may play a role in breast cancer.2

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1. The absorbance (OD) of calibrator 0 ug/ml should be > 1.3

12. Risk Analysis

criteria should be met:

In order for the assay results to be considered valid the following

*The represented in Example 1 and Figure 1 is for illustration only

10. Accurate and precise pipetting, as well as following the exact

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

5. The addition of substrate solution initiates a kinetic reaction,

3. Highly lipemic, hemolyzed or grossly contaminated

2. Patient specimens with DHEA-S concentrations above 8.0

1. The absorbance (OD) of calibrator 0 ug/ml should be > 1.3

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>0.61</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>1.36</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>4.73</td>
</tr>
</tbody>
</table>

4.9% 4.3%

1. It is important that the time of reaction in each well is held

12. Interpretation

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

1. 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 test kits are altered, such as by mixing parts of different kits, or used in lieu of a dose response curve

13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC -

14.3 Accuracy

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.

14.4 Specificity

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.

14.3 Accuracy

The DHEA-S AccuBind® ELISA Test System was compared with a cholesteriminoimmune immunoassay method. Biological specimens from low, normal and relatively high DHEA-S levels were used (The values ranged from 0.2 ug/ml – 7.7 ug/ml). The total number of such specimens was 77. The least square regression equation and the correlation coefficient were computed for this DHEA-S EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (x)</th>
<th>Least Square Regression Analysis</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monobind (y)</td>
<td>1.12</td>
<td>y = 0.1448 + 0.898x</td>
<td>0.983</td>
</tr>
<tr>
<td>Reference (X)</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of percent values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analyst using the method with a population indigenous to the area in which the laboratory is located.

1.10 G.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 ug/ml 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 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. Patient specimens with DHEA-S concentrations above 8.0 ug/ml may be diluted (1/5, 1/10 or higher) with DHEA-S '0' calibrator and re-assayed. The sample’s concentration is obtained by multiplying the result by the dilution factor.

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

Any deviation from Monobind® IFU may yield inaccurate results.

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

12. 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.

13. 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

The least square regression equation and correlation coefficient indicates excellent method agreement.

14.2 Sensitivity

The DHEA-S AccuBind® ELISA Test System was determined by analyses on specimens from low, normal and relatively high DHEA-S level populations were used (The values ranged from 0.2 ug/ml – 7.7 ug/ml). The total number of such specimens was 77. The least square regression equation and the correlation coefficient were computed for this DHEA-S EIA in comparison with the reference method. The data obtained is displayed in Table 4.

14.3 Accuracy

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.

14.4 Specificity

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

15.0 REFERENCES


Revision: 5 Date: 2019-Jul-16 DCO: 1355

TABLE 3

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9.5% 3.4%

TABLE 2

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</thead>
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<td>0.66</td>
</tr>
<tr>
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<td>16</td>
<td>1.14</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>4.84</td>
</tr>
</tbody>
</table>

9.9% 4.9% 4.3%