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 native antigen, a competition 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:

\[
\frac{Eag + A}{k_a} = \frac{EagA}{k_{a}} + \frac{A}{k_{-a}}
\]

where:

- \(Eag\) = Biotinylated-Enzyme-Antigen Conjugate (Constant Quantity)
- \(A\) = Native Antigen (Variable Quantity)
- \(Eag\) = Enzyme-Antigen Conjugate (Constant Quantity)
- \(Ka\) = Rate Constant of Association
- \(K_{-a}\) = Rate Constant of Dissociation
- \(K = \frac{k_a}{k_{-a}}\) = 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 antigen fraction after decantation or aspiration.\(Ag_{A} = \frac{EagA}{k_{a}}\) = Enzyme-antigen Complex, \(Ag_{A} = \frac{EagA}{k_{a}}\) = antibody and \(Ag_{A} = \frac{EagA}{k_{a}}\) = native antigen.\(Ka\) is the constant of association and \(K_{-a}\) is the constant of dissociation. The addition of DHEA to a microwell containing biotinylated antibody will result in the formation of a sandwich with a complex between the antibody and the streptavidin.

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different standard curves, the concentration of antigen can be determined. A dose response curve can be generated from which the antigen concentration of an unknown specimen can be ascertained.

4.0 REAGENTS

A. DHEA Calibrators – 1mL/vial – Ions A-F

Each vial of serum reference for DHEA is used at concentrations of 0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 (ng/ml) in ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expected to give a linear response in molar concentration by multiplying by 3.47.\(\times\) for example: 1ng/ml x 3.47 = 3.47 ng/ml.

B. DHEA Enzyme Reagent – 6.0 ml/vial – Icon

One (1) vial containing DHEA (Analogs)-horseradish peroxidase (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

C. DHEA Biotin Reagent – 6.0 ml / Icon

One (1) vial containing anti-DHEA biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C.

D. Light Reaction Wells – 96 wells – Icon

Clean, white microwell coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution – 20ml/vial – Icon

One (1) vial containing wash solution in buffered saline. A preservative has been added. Store at 2-8°C.

F. Signal Reagent A – 7.0ml/vial – Icon

Store at 2-8°C. Store at 2-8°C.

G. Signal Reagent B – 7.0ml/vial – Icon

One (1) vial containing hydrogen peroxide (H2O2) in 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 are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microwell.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025 & 0.050 ml (25 & 50 µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.500 ml (100 & 350 µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microtube (1 ml) and vials (5 ml).
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microfiber cloth for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
10. Quality control materials.

5.0 PRECAUTIONS

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. Antigen control and antibody control 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 and Biomedical Laboratories, 2nd Edition, 1988, HHS Publication No. EDC (88-8305).

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 heparinised plasma in accordance with the usual precautions in the collection of venipuncture samples. The blood shall be collected in a red top tube (or without gel additives) venipuncture tube or for plasma use evacuated tube containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Sample may be refrigerated at 2-8°C for a maximum period of five (5) days from collection if the specimen can be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to thirty (30) days. Avoid use of contaminated dishes. Avoid repeated freezing and thawing as assayed in duplicate, 0.050 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

For In Vitro Diagnostic Use

Each laboratory should adopt controls at levels in the low, normal and high range of the intended assay for quality assurance. These controls should be treated as unknowns and values determined in every test procedure. Control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from background reading will 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

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum 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 microwells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25 µl) of the appropriate serum reference calibrator, control or specimen into each well.
3. Add 0.050 ml (50 µl) of the DHEA Enzyme Reagent to all wells.
4. Swirl the microwell gently for 20-30 seconds to mix.
5. Add 0.050 ml (50 µl) of Anti-DHEA Biotin Reagent to all wells.
6. Swirl the microwell gently for 20-30 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microwell by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and dry) or aspirate. Repeat four (4) additional times for a total of five (5) 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 disperse the wash. Decant the wash and repeat two (2) additional times.
10. Add 0.100 ml (100 µl) of working signal reagent solution to all wells (See Reagent Preparation Section). Allow the reagents in the same order to minimize reaction time and non-specific binding.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

Incubate at room temperature for five (5) minutes in the dark.

12. Add 0.050 ml (50 µl) of DHEA Biotin Reagent for 20-30 seconds in each well.

The results should be read within thirty (30) minutes of added signal reagents. Note: Dilute the samples suspected of concentrations higher than 30ng/ml 1:5 with DHEA 0’ nmol/gm calibrator

9.0.1 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of progesterone in the specimen.

1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs versus the serum reference versus the corresponding Progesterone concentration in ng/ml on linear loggraph paper.
3. Draw the best-fit curve through the plotted points.
4. Determine the concentration of DHEA for an unknown, locate the average RLUs 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 dilutions of the unknown may be averaged as indicated). In the following example, the average RLUs (62250) of the unknown intersects the calibration curve at 2.430 ng/ml DHEA concentration (see Figure).

Note: Computer data reduction software designed for CLIA Assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

Note: Do not use reagents that are contaminated or have bacteria growth.
The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

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 signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same 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. 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.
9. 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.
10. 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.
11. 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. 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;34:27-33). 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 range and assays requirements.
5. If test kits are altered, such as by mixing parts of different kits, measurements and interpretation of results must be performed by a skilled individual or trained professional.
6. 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.
7. Clinically, a DHEA 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 expected ranges for the DHEA Acculite® CLIA Test System are detailed in Table 1.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the DHEA Acculite® CLIA Test System was evaluated by analyzing results on three different levels of pool control sera. The mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Within Assay Precision (Values in ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>N</td>
</tr>
<tr>
<td>Low</td>
<td>10</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
</tr>
<tr>
<td>High</td>
<td>20</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The DHEA Acculite® CLIA Test System has a sensitivity of 0.15ng/ml. 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.

14.3. 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.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA</td>
<td>100.000</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>0.044</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.056</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>0.044</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.001</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>0.070</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.002</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>0.007</td>
</tr>
<tr>
<td>Estradiol</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

15.0 REFERENCES