Monobind Inc.
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AccuBind
ELISA Microwells

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 a relatively low affinity constant for sex hormone binding globulin (SHBG), the bioactivity at the cell level 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 acne, 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 sulfate derivative, which has much longer half life.[4,5] 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,[3] while elevated levels occur in several conditions; including virilizing adrenal adenoma and carcinoma,[7] 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies[2,6] and some cases of female hirsutism.[2] Since very little DHEA is produced by the gonads,[2,3] 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 and structurally related 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 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 followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated x-DHEA IgG Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

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

$Ab_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

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.

4.0 REAGENTS

Materials Provided:

A. DHEA Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for DHEA at concentrations of 0 (**A**), 0.5 (**B**), 2.0 (**C**), 5.0 (**D**), 10.0 (**E**) and 30.0 (**F**) in ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.47.
For example: 1ng/ml x 3.47 = 3.47 μM/L

B. DHEA Enzyme Reagent – 6ml/vial – Icon
One (1) vial of DHEA (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

C. DHEA Biotin Reagent – 6ml/vial - Icon ∇
One (1) vial of reagent contains anti-DHEA biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1.0 μg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

F. Substrate Solution – 12ml/vial - Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (0.5M H_2SO_4). Store at 2-8°C.

H. Product Instructions.

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 componenet stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate**.**

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50μl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350μl) 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. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

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 capable of transmitting disease. 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, 1988, HHS Publication No. (CDC) 88-8395.

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 heparanised plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal 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 anti-coagulants (for serum) or evacuated tube(s) 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.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 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 five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20oC 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 low, normal 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 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 established performance can indicate unnoticed 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: 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. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
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 0.050ml (50μl) of Anti- DHEA Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 60 minutes 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. Decant the wash and repeat two (2) additional times.**
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 fifteen (15) 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

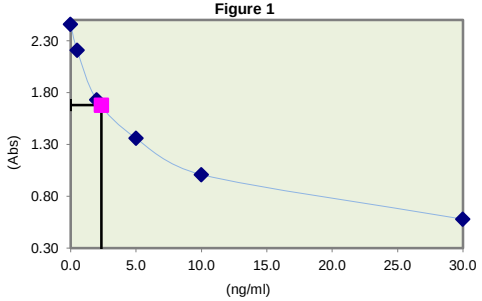
A dose response curve is used to ascertain the concentration of DHEA in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding DHEA concentration in ug/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. 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 on the curve, and read the concentration (in ug/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 dose response curve at (2.36ng/ml) DHEA concentration (See Figure 1).

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.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.484	2.463	0.0
	B1	2.442		
Cal B	C1	2.254	2.209	0.5
	D1	2.164		
Cal C	E1	1.770	1.727	2.0
	F1	1.684		
Cal D	G1	1.423	1.379	5.0
	H1	1.336		
Cal E	A2	1.029	1.006	10.0
	B2	0.983		
Cal F	C2	0.592	0.577	30.0
	D2	0.561		
Cont 1	G2	2.108	2.150	0.62
	H2	2.193		
Pat# 1	A3	1.707	1.680	2.36
	B3	1.651		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 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 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. 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.
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. 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. 1988:3427-33).* 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, 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 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.

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.

TABLE I
Expected Values for DHEA AccuBind® ELISA Test System

	(ng/ml)
Male	1.8 – 12.5
Female	1.3 – 9.8

It is important to keep in mind that establishment of a range of 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 analysts using the method with a population indigenous to the area in which the laboratory is located.

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.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	16	0.72	0.07	9.8%
Normal	16	2.72	0.14	4.9%
High	16	6.73	0.34	6.0%

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	10	0.69	0.07	10.7%
Normal	10	2.85	0.18	6.3%
High	10	6.89	0.41	6.0%

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

14.2 Sensitivity

The DHEA AccuBind® ELISA Test System has a sensitivity of 0.10ng/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.

Substance	Cross Reactivity
DHEA	100.000
DHEA-S	0.004
Androstenedione	0.056
Corticosterone	0.004
Cortisol	0.001
Pregnenolone	0.070
Testosterone	0.002
Dihydrotestosterone	0.007
Estriol	<0.001
Estradiol	<0.001
Estrone	<0.001

15.0 REFERENCES

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MP7425 Product Code: 7425-*300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE

EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

2°-8° — Temperature Limitation Storage Condition (2-8° C)

Consult Instructions for Use

REF — Catalogue Number

Σ — Contains Sufficient Test for Σ

LOT — Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE — European Conformity