MP Biomedicals
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Dehydroepiandrosterone (DHEA)
ChLIA Kit
Catalog No.: 07M7475A

INTENDED USE:

The MP Biomedicals DHEA ChLIA Kit is intended to be used for the quantitative determination of Dehydroepiandrosterone (DHEA) concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence (ChLIA). This test is for in vitro diagnostic use only.

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 (MP Biomedicals Product 07M5175A 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 MP Biomedicals DHEA ELISA 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.

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-S IgG Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}Ag\ Ab_{Btn}$ = 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 affects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin_{CW}} \Rightarrow \underline{\text{immobilized complex}}$
$\underline{Streptavidin_{CW}}$ = 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.

REAGENTS

Materials Provided:

A. DHEA Calibrators – 1 mL/vial
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: 1 ng/mL x 3.47 = 3.47 μM/L

B. DHEA Enzyme Reagent – 6.0 mL/vial
One (1) vial containing DHEA (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

C. DHEA Biotin Reagent – 6.0 mL
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
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

F. Signal Reagent A – 7.0 mL/vial
One (1) vial contains luminol in a buffer. Store at 2-8°C.

G. Signal Reagent B – 7.0 mL/vial
One (1) vial contains hydrogen peroxide (H_2O_2) 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 microplate**.**

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 & 0.350 mL (100 & 350 μL) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000 μL) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Luminometer.
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.

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.

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. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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 -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 mL (50 μL) of the specimen is required.

REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of Wash Concentrate to 1000 mL with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 mL of A and 1 mL of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

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

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 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 0.025 mL (25 μL) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 mL (50 μL) of the DHEA Enzyme Reagent to all wells.
4. Swirl the microplate 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 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 0.350 mL (350 μL) of wash buffer (see Reagent Preparation Section), decant (tap and blot) 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 dispense the wash. Decant the wash and repeat four (4) additional times.**
10. Add 0.100 mL (100 μL) of working signal reagent solution to all wells (See Reagent Preparation Section). **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 five (5) minutes in the dark
12. Read the relative light units (RLUs) in each well with a Chemiluminescence microplate reader for 0.5-1.0 seconds. **The results should be read within thirty (30) minutes of adding the working signal reagent.**

Note: Dilute the samples suspected of concentrations higher than 30 ng/mL 1:5 with DHEA '0' ng/mL calibrator.

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control records should be maintained and used to monitor batch to batch consistency.

CALCULATION OF RESULTS

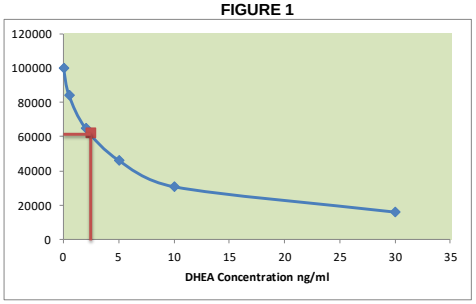
A dose response curve is used to ascertain the concentration of progesterone in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference versus the corresponding Progesterone concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To 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 duplicates 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 Figure1).

Note: Computer data reduction software designed for ChLIA 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	RLU's (A)	Mean RLU's (B)	Value (ng/mL)
Cal A	A1	100456	100000	0.0
	B1	99544		
Cal B	C1	84221	84174	0.5
	D1	84127		
Cal C	E1	65331	64940	2.0
	F1	64550		
Cal D	G1	46790	46162	5.0
	H1	45535		
Cal E	A2	30515	30844	10.0
	B2	31173		
Cal F	C2	16102	16062	30.0
	D2	16021		
Cont 1	G2	58395	57703	3.158
	H2	57011		
Pat# 1	A3	62301	62250	2.430
	B3	62198		

FIGURE 1



***** The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the A calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

LIMITATIONS OF PROCEDURE

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 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 intermixing 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 MP Biomedicals' 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.

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, MP Biomedicals 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 value alone is not of diagnostic value** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the DHEA ChLIA test are detailed in Table 1.

TABLE I
Expected Values for the DHEA ChLIA 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.

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the DHEA ChLIA test 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	20	2.992	0.232	7.8
Normal	20	4.759	0.361	7.6
High	20	7.651	0.641	8.4

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

Sample	N	X	σ	C.V.%
Low	10	3.07	0.308	10.0
Normal	10	4.77	0.126	2.7
High	10	7.61	0.109	1.4

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

Sensitivity

The DHEA ChLIA test has a sensitivity of 0.15 ng/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.

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

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