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Datasheet for ABIN649068

Dehydroepiandrosterone Sulfate ELISA Kit

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Overview

Quantity:	96 tests
Target:	Dehydroepiandrosterone Sulfate
Reactivity:	Human
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	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 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.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	A. DHEA-S Calibrators (1ml/vial). Six vials of serum reference for DHEA-S at concentrations of 0 (A), 0. 2 (B), 1. 0 (C), 2. 0 (D), 4. 0 (E) and 8. 0 (F) in (g/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 2. 71. For example: 1 (g/ml x 2. 71 equal 2. 71 (M/LB. DHEA-S Enzyme Reagent (6. 0 ml/vial). One vial of DHEA-S (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing

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Product Details

	matrix with red dye. Store at 2-8°C. C. DHEA-S Biotin Reagent (6. 0 ml). One bottle of reagent contains anti-DHEA-S biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C. D. Streptavidin Coated Plate (96 wells). 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). One vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-30°C. F. Substrate A (7ml/vial). One vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. G. Substrate B (7ml/vial). One vial contains hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. H. Stop Solution (8ml/vial). One vial contains a strong acid (1N HCl). Store at 2-30°C. I. Product Instructions: Note 1: Do not use reagents beyond the kit expiration date. Note 2: Opened reagents are stable for 60 days when stored at 2-8°C. Note 3: Above reagents are for a single 96-well microplate.
Material not included:	1. Pipette capable of delivering 10 ml and 50ml with a precision of better than 1. 5%. 2. Dispenser(s) for repetitive deliveries of 0. 100ml and 0. 350ml 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.

Target Details

Target:	Dehydroepiandrosterone Sulfate
Alternative Name:	Dehydroepiandrosterone sulfate (DHEA-S) (Dehydroepiandrosterone Sulfate Products)
Background:	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 enzymatic conversion of DHEA in adrenal and extradrenal tissues. Due to the presence of a 17-oxo [rather than hydroxyl] group, DHEA-S possesses relatively weak androgenic activity, which for unsulfated DHEA has been estimated at ~10% that of testosterone [1]. However, the bioactivity of DHEA-S may be increased by its relatively high serum concentrations, approximately 100 to 1000-fold higher than DHEA or testosterone, and its weak affinity for sex-hormone binding globulin [2]. The physiologic role of DHEA-S is not well-defined. Serum levels are relatively high in the fetus and neonate, low during childhood, and increase during puberty [3, 4]. Increased levels of DHEA-S during adrenarche may contribute to the development of secondary sexual hair. DHEA-S levels show a progressive decline after the third decade of life

[5]. Unlike DHEA, DHEA-S levels do not show significant diurnal variation, show little day-to-day variation, are not responsive to acute corticotropin administration [4], and do not vary significantly during the normal menstrual cycle [2]. This may be due to the slower metabolic clearance rate of DHEA-S as compared to DHEA [6]. Measurement of serum DHEA-S 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 3beta-hydroxysteroid dehydrogenase deficiencies [2,6] and some cases of female hirsutism [2]. Since very little DHEA-S is produced by the gonads [2, 3], measurement of DHEA-S may aid in the localization of the androgen source in virilizing conditions. Methods for measurement of DHEA-S include gas-liquid chromatography, double-isotope derivative techniques, competitive protein-binding assays, and radioimmunoassay. Although significant cross-reactivity occurs with DHEA, androstenedione and androsterone, the relative concentrations of these competing substances in most normal and pathologic samples predicts a minimal effect on assay performance. DHEA-S ELISA Kits use a specific anti-DHEA-S antibody, and do 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-S 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-S concentration. Intended Use: The Quantitative Determination of Dehydroepiandrosterone Sulfate Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay. 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 greater than 1.3. 2. Four out of six quality control pools should be within the established ranges.

Application Details

Application Notes:	Precautions: 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.
Plate:	Pre-coated
Reagent Preparation:	1. Wash Buffer: Dilute contents of wash solution to 1000ml with distilled or deionized water in a

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	suitable storage container. Diluted buffer can be stored at room temperature (20-27°C) for up to 60 days. 2. Working Substrate Solution - Stable for 1 year: Pour the contents of the amber vial labeled Solution A into the clear vial labeled Solution B. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8 °C. Note: Do not use the working substrate if it looks blue.
Sample Collection:	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. Samples may be refrigerated at 2-8°C for a maximum period of five 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 repetitive freezing and thawing. When assayed in duplicate, 0. 020ml of the specimen is required.
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 printout of the microplate reader. 2. Plot the absorbance for each duplicate serum reference versus the corresponding DHEA-S 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. To determine the concentration of DHEA-S 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).
Restrictions:	For Research Use only

Handling

Handling Advice:	Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C). 1. Format the microplates' wells for each serum reference, 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. 010 ml (10 µl) of the appropriate serum reference, control or specimen into the assigned well. 3. Add 0. 050 ml (50µl) of the DHEA-S Enzyme Reagent to all wells. 4. Swirl the microplate gently for 20-30 seconds to mix. 5. Add 0. 050 ml (50µl) of Anti- DHEA-S Biotin Reagent to all wells. 6. Swirl the microplate gently for 20-30

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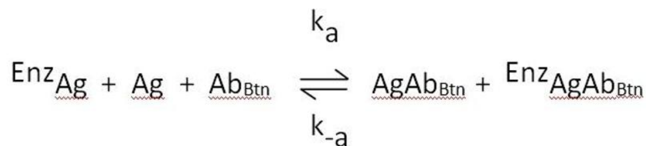
seconds to mix. 7. Cover and incubate for 30 minutes at room temperature. 8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper. 9. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two additional times for a total of three 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 additional times. 10. Add 0. 100 ml (100µl) of working substrate 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 15 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 30 minutes of adding the stop solution. Note: Dilute the samples suspected of concentrations higher than 8. 0 ug/ml 1:5 and 1:10 with DHEA-S 0 µg/ml calibrator or patient serum pools with a known low value for DHEA-S.

Storage:	4 °C/-20 °C

Images



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

Ab_{Btn} = Biotinylated 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

Image 1. The essential reagents required for a 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 equation in Figure 1.

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 (Figure 2).

The enzyme activity in the antibody- bound fraction is inversely proportional to the native antigen concentration.

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Images

By utilizing several different serum references of known antigen con centration, a dose response curve can be generated from which the anti gen concentration of an unknown can be ascertained.