Datasheet for ABIN649069

17a-Hydroxyprogesterone ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	17a-Hydroxyprogesterone
Reactivity:	Human
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	Competitive Enzyme Immunoassay (TYPE 7): 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 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. 17a-OH Progesterone Calibrators (1ml/vial). Six vials of serum reference for 17-OH Progesterone at concentrations of 0 (A), 0. 1 (B), 0. 5 (C), 1. 0 (D), 2. 5 (E), and 10 (F) 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. 03. For example: 1ng/ml x 3. 03 equal 3. 03 nM/LB. 17a-OH Progesterone Enzyme Reagent (1. 0 ml/vial). One vial of 17-OH ProgesterOne (Analog)-

Product Details

	horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with dye. Store at 2-8°C. C. Steroid Conjugate Buffer (7. 0 ml/vial). One vial of reagent contains buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C. D. 17a-OH Progesterone Biotin Reagent (6. 0 ml). One bottle of reagent contains anti-17a-OH Progesterone biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C. E. 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. F. Wash Solution Concentrate (20ml). One vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-30°C. G. Substrate Solution (12ml/vial). One bottle contains tetramethylbenzidine (TMB) and hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. H. Stop Solution (8ml/vial). One vial contains a strong acid (0. 5M H2SO4). 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 25ml 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:	17a-Hydroxyprogesterone
Alternative Name:	17a-hydroxyprogesterone (17a-OHP)
Target Type:	Hormone
Background:	Summary and Explanation of the test: Plasma/Serum concentrations of 17a-hydroxyprogesterone (17a-OHP) are valuable in the initial diagnosis of congenital adrenal hyperplasia (CAH) 1, 2. This common inborn error of metabolism is usually characterized by deficiency in the C21-hydroxylase enzyme system, and necessitates steroid replacement therapy. Adequacy of treatment has been monitored by determining circulating 17a-OHP concentrations3,4. The incidence is roughly estimated to be 1 in 15,000 newborns and can reach as high as 1 in 1480 in native Alaskans. Early diagnosis is valuable to detect CAH in newborns afflicted with the disease, not clinically recognizable but which will lead to life threatening adrenal crisis in the neonatal period and to determine the cause of infants with

Target Details

ambiguous genitalia. Delayed diagnosis may also lead to further virilization in female children, acceleration of skeletal maturation and premature development of secondary sex characteristics in male children. Prompt treatment can save the life of infants and allow afflicted children to attain normal growth. 17P is a steroid produced in the adrenal cortex and the gonads. It is the immediate precursor to 11-desoxycortisol (CpS) which is converted to cortisol. Because CpS is produced by 21-hydroxylation of 17P, measurement of 17P is an indirect indicator of 21-hydroxylase activity. CAH occurs where there is a deficiency of this enzyme. The result is a decrease in the conversion of 17P to CpS which blocks the normal synthesis of cortisol. Due to the feed back mechanism, a decrease in cortisol causes an increase in ACTH secretion resulting in adrenal hyperplasia. As 17P is not being converted, increased concentrations of this steroid will be found. 17P concentration increases during pregnancy in the maternal and fetal blood. After birth, values decline rapidly to reach normal adult values in 2 to 7 days. Thus it is advisable not to collect samples before the 3rd day of life. Premature and sick term infants exhibit 2 to 3 fold 17P values with no CAH disorder. It is suggested that a different cut off be adopted to pre-term and sick infants. In this method, a sample containing 17-OH progesterone is dispensed into a microplate well. An enzyme labeled 17OH progesterone derivative and biotinylated anti-17OH-progesterone are than added. After a suitable incubation, the antibody fraction is separated from unbound enzyme reagent. The employment of several serum references of known 17-OH Progesterone 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 17-OH Progesterone concentration. Intended Use: The Quantitative Determination of 17-OH Progesterone 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 ng/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.

Application Details

Plate:	Pre-coated
Reagent Preparation:	1. Working Enzyme Reagent - Stable for 1 year: Measuree 0. 7 ml of 17-OH Progesterone Enzyme Reagent and add to the vial containing Steroid Conjugate Buffer. Store at 2-8°C. 2. 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 (20-27°C) for up to 60 days. 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 (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 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. 050ml of the specimen is required.
Calculation of Results:	A dose response curve is used to ascertain the concentration of 17-OH Progesterone 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 17-OH Progesterone concentration in ng/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 17-OH Progesterone 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 ng/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. 025 ml (25 µl) of the appropriate serum reference, control or specimen into the assigned well. 3. Add 0. 050 ml (50µl) of working 17a-OH Progesterone Enzyme Reagent to all wells. 4. Swirl the microplate gently for 20-30 seconds to mix. 5. Add 0. 050 ml (50µl) of the 17a-OH Progesterone Biotin Reagent to all wells. 6. Swirl

Handling

	the microplate gently for 20-30 seconds to mix 7. Cover and incubate for 60minutes 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 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 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 30 minutes of adding the stop solution. Note: Dilute the samples suspected of concentrations higher than 20ng/ml 1:1 and 1:5 with 17-OH Progesterone 0 ng/ml calibrator or male patient serum pools with a known low value for 17-OH Progesterone.
Storage:	4 °C/-20 °C