Datasheet for ABIN649056

SERPINA7 ELISA Kit

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Overview

Quantity:	96 tests
Target:	SERPINA7
Reactivity:	Human
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	Immunoenzymometric assay: The essential reagents required for an enzyme immunoassay include high affinity and specificity antibody in, enzyme labeled antigen and the native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated polyclonal anti-TBG antibody. Upon mixing polyclonal biotinylated antibody, the enzyme-labeled antigen and a serum containing the native antigen, a competition results between the native antigen and the enzyme labeled antigen for a limited number of specific binding sites on the antibody. The antigen bound antibody attaches to the surface of the plastic wells because of the biotin label on it and the streptavidin that is present on the plastic. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the excess serum proteins, antibodies and the enzyme labeled antigen are removed via a wash step. 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 values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.
Detection Method:	Colorimetric

Product Details

Components:	A. TBG Calibrator (0. 5 ml/vial). Si vials of references TBG Antigen at levels of 1(A), 4(B), 8(C), 16(D), 32(E) and 64(F) µg/ml. Store at 2-8°C. A Preservative has been added. Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the international reference material (IS 88/638). B. TBG EnzymReagent (5. 5 ml/vial). One vial containing Enzyme (HRP) labeled TBG in buffer, dye, and preservativ Store at 2-8°C. C. Antibody Biotin Reagent (5. 5 ml). One vial of Biotin labeled Anti-TBG polyclonal IgG in buffer, dye and preservatives. Store at 2-8°C. D. Streptavidin Coated Microplate (96 wells)). One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C. E. Wash Solution Concentrate (20 ml). One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C. F. Substrate A (7ml/vial). One bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. G. Substrate B (7ml/vial). One bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. H. Stop Solution (8m/vial). One bottle containing a strong acid (1N HCl). Store at 2-30°C. I. Product Insert. 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 & 50 µl volumes with a precision of better than 1. 5%. 2. Multi-channel dispenser(s) for deliveries of 0. 100ml and 0. 350ml volumes with a precision of better than 1. 5%. 3. Microplate washers or a squeeze bottle (optional). 4. Microplate Reader with 450nm and 620nm wavelength absorbance capability. 5. Absorbent Paper for blotting the microplate wells. 6. Plastic wrap or microplate cover for incubation steps. 7. Vacuum aspirator (optional) for wash steps. 8. Timer. 9. Quality control materials.

Target Details

Target:	SERPINA7
Alternative Name:	TBG (SERPINA7 Products)
Background:	Summary and Explanation of the Test: TBG (Thyroxine Binding Globulin) a 54 kD liver glycoprotein is the principal binding protein for T4 and T3 in circulation. Electrophoretic analyses indicate that T4 is bound, in decreasing order, to TBG, to a T4 binding prealbumin (TBPA) and to albumin. By virtue of its intense affinity for T4, TBG is by far the major determinant of overall binding capacity. The interaction between T4 and its binding proteins conforms to a reversible binding equilibrium in which the majority of the hormone is bound and a very small portion (lower than 0. 05%) is free. T3 is not bound by TBPA and is bound by TBG less firmly than is T4. As a consequence proportion of free T3 is normally 8-10 times greater than T4. Only free (T3/T4) hormones are available to the tissues, therefore the metabolic state

of the patient will correlate more closely with the free than with the total concentration of the hormones. The diagnostic accuracy of the total hormone measurements would be equal to the free hormone if all the patients had similar binding protein concentrations. Unfortunately, serum TBG abnormalities that distort the total: free relationship, are commonly encountered in clinical practice. Additionally the presence of antibodies to thyroid hormones, in some patients, render total hormone measurements unreliable. Considerable confusion still exists regarding the validity of free hormone testing. There is controversy regarding the clinical utility of free hormone testing in conditions associated with binding protein abnormalities of pregnancy and non-thyroidal illness. Methods that are sensitive to albumin concentrations, the effect of certain drugs, high free fatty acid and levels of hormones binding inhibitors are considered inadequate by some researchers. However, the techniques for physically separating the exceedingly small amounts of free hormones from the dominant protein bound moiety are too technically demanding, inconvenient and expensive for a routine clinical laboratory. Such methods that employ equilibrium dialysis, ultrafilteration and gel-filtration are typically used by researchers. In routine analysis the clinical laboratories rely on direct measurements of free and total hormones and their binding proteins, mainly TBG. Based on their serum concentrations, familial TBG variants are divided into four major categories: excess, normal, partial deficiency and complete absence. The studies show that estrogens – pregnancy and oral contraceptives – acute intermittent porphyria and chronic liver disease increase TBG concentrations while, androgenic and anabolic steroids, large doses of glucocorticoids and nephrosis decreases TBG levels. In this method, TBG calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated polyclonal antibody (highly specific for TBG) and enzyme labeled TBG are added, in sequence, and the reactants mixed. Reaction between the TBG antibodies, enzyme labeled TBG and native TBG forms a complex that binds with the streptavidin coated to the well. After the completion of the required incubation period, the excess enzyme conjugate is separated from the bound fraction via a wash step. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known TBG levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with TBG concentration. Intended Use: The Quantitative Determination of TBG Thyroxine Binding Globulin concentration in Human Serum, Plasma or Whole Blood by a Microplate Enzyme Labeled Immunoassay. Q. C. Paramters: In order for the assay results to be considered valid the following criteria should be met. The absorbance (OD) of calibrator F should be greater than 1.3. 2. Four out of six quality control pools should be within the established ranges. LIMITATIONS OF PROCEDURE

Target Details

Pathways:	Hormone Transport

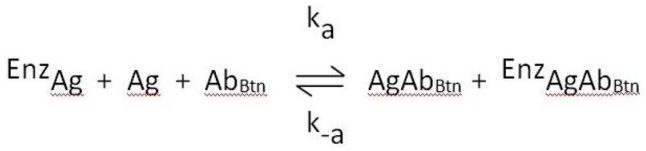
Application Details

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 licensed reagents. 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.
Sample Volume:	10 µL
Reagent Preparation:	1. Wash Buffer: Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days. 2. Working Substrate Solution: 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°. Note: Do not use the working substrate if it looks blue.
Sample Collection:	The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum 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 TBG 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 TBG concentration in µg/ml on linear graph paper. 3. Draw the best-fit curve through the plotted points. 4. To determine the concentration of TBG 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. 010 ml (10 µl) of the appropriate serum reference, diluted control or specimen into the assigned wells. 3. Add 0. 050 ml (50 µl) of the TBG enzyme reagent to each well. Mix well the contents of the microwells. It is very important to dispense all reagents close to the bottom of the coated well. 4. Add 0. 050 ml (50 µl) of the biotin antibody reagent to each well. 5. Swirl the microplate gently for 20-30 seconds to mix and cover. 5. Incubate 30 minutes at room temperature. 6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper. 7. 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. 8. Add 0. 100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). 9. Incubate at room temperature for 15 minutes. 10. Add 0. 050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. 11. Read the absorbance in each well at 450nm using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within 30 minutes of adding the stop solution. Note: Always add reagents in the same order to minimizereaction time differences between wells.
Storage:	4 °C/-20 °C

Images



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. 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.