Datasheet for ABIN649024

TPO Ab ELISA Kit

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Overview

Quantity:	96 tests
Target:	TPO Ab
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	A Sequential ELISA Method (TYPE 1): The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. 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 thyroid peroxidase antigen. Upon mixing the biotinylated antigen and a serum containing the autoantibody, a reaction results between the antigen and the antibody to form an immune-complex. The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	A. xAnti-TPO Calibrators (1. 0 ml/vial). Six vials of references for anti-TPO at levels of 0 (A), 25 (B), 50 (C), 100 (D), 250 (E) and 500 (F) IU/ml. Store at 2-8°C. A preservative has been added.

Product Details

	Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the Medical Research Council (MRC) International Standard 66/2387 for anti thyroid microsome. B. Thyroid PeroxidaseTPO Biotin ConjugateReagent (11ml - 13ml/vial). One vial of biotinylated thyroid peroxidase antigen stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C. C. Enzyme-antigen Conjugate xAnti-TPO Enzyme Reagent (11ml - 13ml/vial). One vial of anti-human IgG-horseradish peroxidase (HRP) conjugate stabilized in a buffering matrix. A preservative has been added. Store at 2-8°C. D. Streptavidin Coated Microplate Plate (96 wells). One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C. Serum Diluent Concentrate (20ml). One vial of serum diluent concentrate containing buffer salts and a yellow dye. Store at 2-8°C. E. Serum Diluent Concentrate (20ml). One vial of serumof serum diluent containing buffer salts and a dye. Store at 2-8°C. (See note 3). F. Wash Solution Concentrate (20ml). One vial containing a surfactant in phosphate buffered saline. A preservative has been added. Store at 2-8°C. G. Substrate A (7. 0ml/vial). One bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. H. Substrate B (7. 0ml/vial). One bottle containingbottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C. I. Stop Solution (86. 0ml/vial). One bottle containing a strong acid (1N HCl). Store at 2-8°C. J. 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. 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. The serum diluent concentrate can cloud or show precipitation when stored at 2-8°C. This is normal as the reagent is supplied highly concentrated. Please warm to room temperature to solubilize any precipitant before dilution.
Material not included:	1. Pipettes capable of delivering 10, 25µl & 50µl volumes with a precision of better than 1. 5%. 2. Dispenser(s) for repetitive deliveries of 0. 100ml and 0. 3500ml 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. Test tube (s) for patient dilution. 9. Timer. 10. Quality control materials.

Target Details

Target:	TPO Ab
Alternative Name:	Antibodies to thyroid peroxidase (TPO) (TPO Ab Products)

Target Details

Target Type:	Antibody, Antibody, Antibody
Background:	Summary and Explanation of the test: Antibodies to thyroid peroxidase have been shown to be characteristically present from patients with Hashimoto thyroiditis (95%), idiopathic myedema (90%) and Graves Disease (80%). 1. In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction. 2. This dysfunction has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Measurements of antibodies to TPO have been done, in the past, by Passive Hemaglutination (PHA). PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to TPO. In addition, the results are quantitated by a spectrophotometer, which eliminates subjective interpretation. The microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, diluted patient specimen, or control is first added to a microplate well. Biotinylated Thyroid Peroxidase Antigen (TPO) is added, then the reactants are mixed. A reaction results between the autoantibodies to TPO and the biotinylated TPO to form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin. After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate to produce color. The employment of several serum references of known antibody activity permits construction of a graph of enzyme and antibody activities. From comparison to the dose response curve, an unknown specimen's enzyme activity can be correlated with auto-immuneautoimmune antibody level. Intended Use: The Quantitative Determination of Thyroid Peroxidase (TPO) Autoantibodies in Human Serum or Plasma by a Microplate Enzyme Immunoassay. Measurements of TPO autoantibodies may aid in the diagnosis of certain thyroid diseases such as Hashimoto's and Grave's as well as nontoxic goiter and cancer of the thyroid. 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/dlF 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 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.
Comment:	Dilution of Sample: 1-100
Sample Volume:	25 µL
Plate:	Pre-coated
Reagent Preparation:	1. Serum Diluent: Dilute the serum diluent concentrate to 200ml in a suitable container with distilled or deionized water. Store at 2-8°C. 2. Wash Buffer: Dilute contents of Wash concentrate Concentratesolution to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27 °C for up to 60 days. 3. 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. 3. 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 °C. Pour the contents of the vial labeled Solution A into the vial labeled Solution B. Mix and store at 2-8°C. Use within 60 days. Or for longer periods of usage determine the amount of reagent needed and prepare by mixing equal portions of Substrate A and Substrate B in a suitable container. For example, add 1ml of A and 1ml of B per two eight well strips (A slight excess of solution is made. Discard the unused portion). Note: Do not use the working substrate if it looks blue. 4. Patient Sample Dilution (1/100): Dispense 0. 010ml (10µl) of each patient specimen into 1ml of serum diluent. Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to 48 hours.
Sample Collection:	The specimens shall be blood, serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established 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 (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot fopr 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 samples(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0. 05-100ml of the specimen is required. Samples may be refrigerated at 2-8°C for a maximum period of five days.

Application Details

	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. 100ml of the specimen is required.
Calculation of Results:	A reference curve is used to ascertain the concentration of anti-TPO 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 anti-TPO activity in IU/ml on linear graph paper. 3. Draw the best-fit curve through the plotted points. 4. To determine the level of anti-TPO activity 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 IU/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 (1. 323 IU/ml) intersects the dose response curve at 200 IU/ml anti-TPO concentration. Note: Computer data reduction software designed for IEMA (ELISA) assays may also be used for the data reduction to obtain the concentration of the specimen.
Restrictions:	For Research Use only

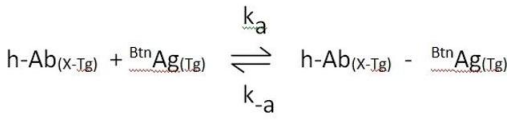
Handling

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 (25µl) of the appropriate serum reference, control or diluted patient specimen into the assigned well. 3. Add 0. 100 ml (100µl) of the TPO Biotinylated Conjugate Solution. Biotin Reagent. 4. Swirl the microplate gently for 20-30 seconds to mix and cover. 5. Incubate 60 minutes at room temperature. 6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper. 7. Add 3500µl of wash buffer (see Reagent Preparation Section), decant (blot and tap) 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 the x-TPO Eenzyme anti-h-IgG Conjugate SolutionReagent to all wells. Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION. 9. Swirl the microplate gently, cover and incubate for 30 minutes at room temperature. Swirl the microplate gently, cover and incubate for 30 minutes at room temperature. 10. Repeat steps (6 & 7) as explained above. 11. Add 0. 100 ml (100µl) of Working

Handling

	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. 12. Incubate at room temperature for 15 minutes. 13. Add 0. 050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells. 14. 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.
Storage:	4 °C/-20 °C

Images



$^{Btn}Ag_{(Tg)}$ = Biotinylated Antigen (Constant Quantity)
$h\text{-}Ab_{(X\text{-}Tg)}$= Human Auto-Antibody (Variable Quantity)
$Ab_{(X\text{-}Tg)}\text{-}{}^{Btn}Ag_{(Tg)}$ = Immune Complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Image 1. The reagents required for the sequential ELISA assay include immobilized antigen, circulating autoantibody and enzyme-linked species-specific antibody. 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 thyroglobulin antigen.

Upon mixing biotinylated antigen and a serum containing the autoantibody, reaction results between the antigen and the antibody to form an immune-complex. The interaction is illustrated by the equation in Figure 1.

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antigen. This interaction is illustrated in Figure 2.

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibody (anti-h-IgG) is then added to the microwells. This conjugates binds to the immune complex that formed (Figure 3).

The anti-h-IgG enzyme conjugate that binds to the immune complex in a second incubation is separated from

unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing several different serum references of known antibody activity, a reference curve can be generated from which the antibody activity of an unknown can be ascertained.