Datasheet for ABIN1305177

Anti-TPO IgG ELISA Kit

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Overview

Quantity:	96 tests
Target:	Anti-TPO IgG (TPO IgG)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0 U/mL - 720 U/mL
Minimum Detection Limit:	1 U/mL
Application:	ELISA

Product Details

Purpose:	This microplate based ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human anti-TPO autoantibody (IgG) level in serum. The presence of this autoantibody together with clinical findings and other laboratory tests is a useful tool in the aid of diagnosis of autoimmune thyroid disease.
Brand:	ED™
Sample Type:	Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This EIA is specific for the measurement of human anti-TPO IgG. No cross-reactivity to other autoantibodies has been observed.
Characteristics:	This high sensitive ED™ anti-TPO autoantibody ELISA kit was developed with proprietary technology that leads to a very low reaction background in normal population and thus would

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	increase the clinical diagnostic sensitivity and specificity.
Components:	1. Streptavidin Coated Microplate Two bottles each contains 30 mL phosphate buffer with protein stabilizers and preservative. The reagent is ready to use. This reagent should be stored at 2-8 °C and is stable until the expiration date on the kit box.
Material not included:	1. Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc. 2. Repeating dispenser suitable for delivering 100 µL. 3. Disposable pipette tips suitable for above volume dispensing. 4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes. 5. Disposable plastic 1000 mL bottle with caps. 6. Aluminum foil 7. Deionized or distilled water. 8. Plastic microtiter well cover or polyethylene film. 9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system. 10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Target Details

Target:	Anti-TPO IgG (TPO IgG)
Alternative Name:	Anti-TPO IgG Antibody (TPO IgG Products)
Target Type:	Antibody
Background:	It is a routine practice of measuring serum autoantibodies to thyroglobulin (Tg) and microsomal (TPO) for aid in detecting and monitoring autoimmune thyroid disease. Serum anti-TPO autoantibody and anti-Tg autoantibody are found to be well correlating with histological changes in Harshimoto's thyroiditis. Clinically, positive anti-TPO autoantibody is detected in patients with chronic thyroiditis (70-90 %), primary hypothyroidism (~60 %), thyrotoxicosis (~50 %) and thyroid tumors (~17 %), however, anti-Tg autoantibody is mainly identified in patients with Harshimoto's thyroiditis and Graves' disease (40-70 %). Although ELISA technology has applied to detecting these autoantibodies, the high background in normal population would decrease the clinical diagnostic sensitivity and specificity.

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Sample Volume:	10 µL
Assay Time:	4 h

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Plate:	Pre-coated
Protocol:	This ELISA is designed, developed and produced for the quantitative measurement of human anti-TPO IgG level in test sample. The assay utilizes the streptavidin coated microplate based enzyme immunoassay technique. Assay calibrators, controls and pre-diluted human serum samples containing anti-TPO IgG are added to microtiter wells of microplate that was coated with high affinity streptavidin on its wall. The autoantibody reaction will not start until the addition of a biotinylated human TPO antigen. After the first incubation period, the unbound protein matrix is removed in the subsequent washing step. A horseradish peroxidase conjugated rabbit anti-human IgG subclass specific antibody (tracer antibody) is added to each well. After an incubation period an immunocomplex of solid-phase bound biotin-TPO human anti-TPO IgG HRP-conjugated tracer antibody is formed if there is human anti-TPO IgG autoantibody present in the test sample. The unbound tracer antibody is removed in the subsequent washing step. HRP-conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the human IgG on the wall of the microtiter well is directly proportional to the amount of human anti-TPO IgG autoantibody level in the sample. Plotting the absorbance versus the respective human anti-TPO IgG autoantibody concentration for each calibrator on point-to-point or 4-parameter fit generates a calibrator curve. The concentration of human anti-TPO IgG autoantibody in test samples is determined directly from this calibrator curve.
Reagent Preparation:	(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged. (2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.
Sample Collection:	Only 10 µL of human serum is required for anti-TPO autoantibody measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 ? 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 ? 8 C up to 48 hours and at -20 °C or below for long-term storage until measurement.
Sample Preparation:	(1) Label a test tube (12x75 mm). (2) Add 1 mL of the diluent into each tube. Pipet 10 µL of patient serum sample to the tube and mix well.

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Assay Procedure:	(1) Place a sufficient number of streptavidin coated microwell strips in a holder to run anti-TPO hIgG calibrators, controls and pre-diluted unknown samples in duplicate. (2) Test Configuration (3) Add 25 µL of calibrators , controls and diluted patient serum samples into the designated microwell (4) Add 100 µL of biotinylated TPO solution into each well. (5) Cover the plate with one plate sealer. the plate with one plate sealer. (6) Incubate plate at room temperature for 1 hour. (7) Prepare Anti-hIgG Tracer Antibody Working Solution by 1:21 fold dilution of the Tracer Antibody with the Tracer Antibody Diluent . For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of the Tracer Antibody in a clean test tube. (8) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (9) Add 100 µL of above diluted tracer antibody working solution to each of the wells. (10) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light. (11) Incubate plate at room temperature for 30 minutes. (12) Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used. (13) Add 100 µL of ELISA HRP Substrate into each of the wells. (14) Cover the plate with a new plate sealer and also with aluminum foil to avoid exposure to light. (15) Incubate plate at room temperature for 20 minutes (16) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution into each of the wells. Mix gently. (17) Read the absorbance at 450 nm within 10 minutes in a microplate reader.
Calculation of Results:	1. Calculate the average absorbance for each pair of duplicate test results. 2. Subtract the average absorbance of the calibrator 1 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance. 3. The calibrator curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. The anti-TPO hIgG concentrations for the controls and samples are read directly from the calibrator curve using their respective corrected absorbance. If log-log graph paper or computer assisted data reduction program utilizing logarithmic transformation are

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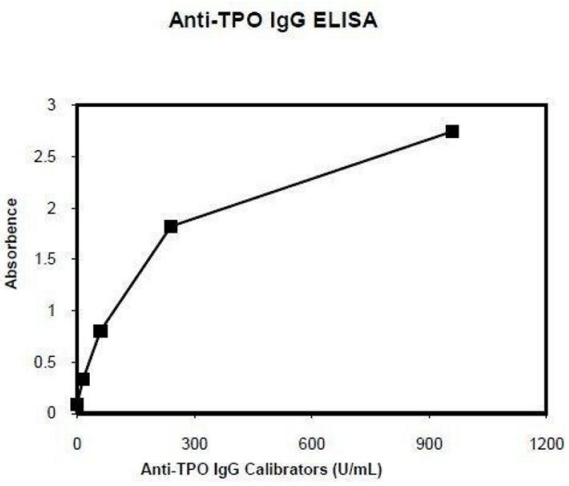
	used, sample having corrected absorbance between the level 2 calibrator and the next highest calibrator should be calculated by the formula:
Assay Precision:	The intra-assay precision is validated by measuring two samples in a single assay with 20 replicate determinations. The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays.
Restrictions:	For Research Use only

Handling

Precaution of Use:	The reagents must be used in research laboratory and are for research use only. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices
Storage:	4 °C

Images



ELISA

Image 1.