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User's Manual

Human Anti-TPO IgG ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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.

General Description

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. This high sensitive anti-TPO IgG ELISA kit was developed with proprietary technology that leads to a very low reaction background in normal population and thus would increase the clinical diagnostic sensitivity and specificity.

Principles of Testing

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 biotinTPO – 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.

Reagents And Materials Provided

1. Streptavidin Coated Microplate

One microplate with 12 x 8 strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil Ziploc bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. Biotinylated TPO

One vial containing 10 ml of ready-to-use biotinylated human TPO solution in a stabilized matrix. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. Anti-hIgG Tracer Antibody

One vial containing 0.6 mL concentrated horseradish peroxidase (HRP) conjugated anti-human IgG tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. Tracer Antibody Diluent

One vial containing 12 mL ready-to-use buffer. It should be only used for antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. ELISA Wash Concentrate

One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mix well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a nonazide preservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. ELISA HRP Substrate

One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. ELISA Stop Solution

One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

8. Anti-TPO hIgG Calibrators

Five vials each contain anti-TPO IgG type autoantibody in a liquid bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration for each calibrator. After the first use, the calibrators should be stored at -20°C or below for long-term storage.

9. Anti-TPO hIgG Controls

Two vials each contain anti-TPO IgG type autoantibody in a liquid bovine serum albumin-based matrix with a non azide preservative. Refer to vials for exact concentration range for each control. After the first use, the calibrators should be stored at -20°C or below for long-term storage.

10. Patient Sample Diluent

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.

Materials Required But Not Supplied

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 (semiautomatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Specimen Collection And Preparation

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.

Assay Procedure

Patient Sample Preparation

Patient sample needs to be diluted 1:101 with Patient Sample Diluent before being measured.

(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.

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.

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

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 2
B	STD 1	STD 5	SAMPLE 2
C	STD 2	C 1	SAMPLE 3
D	STD 2	C 1	SAMPLE 3
E	STD 3	C 2	SAMPLE 4
F	STD 3	C 2	SAMPLE 5
G	STD 4	SAMPLE 1	
H	STD 4	SAMPLE 1	

(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.

(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 µ L 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.

Procedure notes

(1) It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.

(2) Keep light-sensitive reagents in the original amber bottles.

(3) Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.

(4) Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility

of the test.

(5) Incubation times or temperatures other than those stated in this insert may affect the results.

(6) Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.

(7) All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

Quality Control

To assure the validity of the results each assay should include adequate controls with known anti-TPO IgG levels. We recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

Interpretation 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-topoint 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 used, sample having corrected absorbance between the level 2 calibrator and the next highest calibrator should be calculated by the formula:

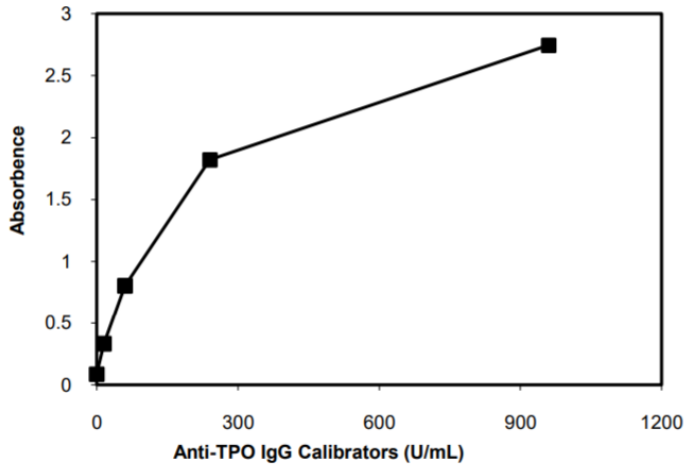
$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2}^{nd}\text{ STD)}} \times \text{Value of the 2}^{nd}\text{ STD}$$

Typical Standard Curve

A typical absorbance data and the resulting calibrator curve from human anti-TPO IgG ELISA are represented. This curve should not be used in lieu of calibrator curve run with each assay.

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 U/mL	0.077 0.095	0.086	0.000	
15 U/mL	0.315 0.342	0.329	0.243	
60 U/mL	0.807 0.799	0.803	0.717	
240 U/mL	1.768 1.874	1.821	1.735	
960 U/mL	2.735 2.756	2.745	2.659	
Control 1	0.198 0.212	0.205	0.119	7.36 U/mL
Control 2	1.052 1.057	1.055	0.969	104.46 U/mL

Anti-TPO IgG ELISA



Reference Values

Serum from 128 normal adults, as well as 60 patients with thyroid diseases were measured with this kit. The following is a guide to interpretation of results. Because the prevalence of human anti-TPO IgG antibodies may vary depending on a number of factors such as age, gender, geographical location, race, type of test used and clinical history of individual patients, it is strongly recommended that each laboratory should establish its own "normal" range based on populations encountered.

Unit Value	Interpretation
< 20 U/mL	Negative
20 – 35 U/mL	Borderline
> 35 U/mL	Positive

Precision

The intra-assay precision is validated by measuring two samples in a single assay with 20 replicate determinations.

Mean Anti-TPO IgG Value (U/mL)	CV (%)
7.5	6.4
102.6	4.9

The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays.

Mean Anti-TPO IgG Value (U/mL)	CV (%)
7.3	7.2
105.9	5.6

Detection Range

0 - 960 U / mL

Sensitivity

The sensitivity of this anti-TPO IgG ELISA Kit as determined by the 95% confidence limit on 20 duplicate determination of zero calibrator is about 1 U/mL.

Specificity

This kit is specific for the measurement of human anti-TPO IgG. No cross-reactivity to other autoantibodies has been observed.

Linearity

Two human serum samples were diluted with assay buffer and assayed. The results in the value of U/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Original	68.2	-	-
	1:2	32.6	34.1	96
	1:4	16.2	17.1	95
	1:8	7.9	8.5	93
2	Original	26.8	-	-
	1:2	14.2	13.4	106
	1:4	6.4	6.7	96
	1:8	3.2	3.4	94

Precautions

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.

Limitations

(1) The results obtained with the anti-TPO IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in itself.

(2) Since there is no Gold Standard concentration available for Anti-TPO IgG measurement, the values of assay calibrators were established and calibrated in arbitrary units (U/mL).

(3) For unknown sample value read directly from the assay that is greater than 200 U/mL, it is recommended to measure a further diluted sample for more accurate measurement.

(4) Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.

(5) Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

References

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