TPO IgG ELISA Kit

Catalog Number KA1900

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the quantitative determination of anti-Thyroid Peroxidase antibodies in human serum.

Principle of the Assay

Microtiter plates are coated with human Thyroid Peroxidase. Serum to be tested is diluted and incubated with the precoated plate. In this step TPO specific antibodies are bound to the immobilized human Thyroid Peroxidase. Non specific antibodies are removed by washing. Anti-human IgG conjugated to horseradish peroxidase (HRP) is added and incubated. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex. Unbound conjugate is removed by washing. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in TMB-substrate solution.

General Information

Materials Supplied

List of component

Component	Description	Amount
Dilution Plate	Plate for the samples predilution with Sample diluent 1	1 plate
Wash Buffer concentrate	Concentrate; Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves at 35-39ºC and shaking.	1 x 50 mL
Substrate	ready for use, containing a solution of tetramethylbenzidine (TMB)	1 x 12 mL
Stop Solution	ready for use, containing 0.2 M H2SO4	1 x 15 mL
Calibrator 0*	ready for use	1 x 1 mL
Calibrator 1*	ready for use	1 x 1 mL
Calibrator 2*	ready for use	1 x 1 mL
Calibrator 3*	ready for use	1 x 1 mL
Calibrator 4*	ready for use	1 x 1 mL
TPO-coated microtiter strips	One 96-well (break apart) plate coated with human Thyroid Peroxidase.	1 x 96 wells
Conjugate	Monoclonal antibodies against human IgG, conjugated with HRP enzyme in a protein-stabilized matrix.	1 x 12 mL
Control serum	ready for use. Human serum based. Refer to vial label for acceptable range.	1 x 1 mL
Sample Diluent 1	Buffer that is used for the first samples dilution in preliminary plate before analysis	1 x 12 mL
Sample Diluent 2	Buffer that is used for the second samples dilution in working plate for analysis.	1 x 12 mL

**Note: Exact levels are given on the certificates of analysis on a lot specific basis. The calibrators, human serum based, were calibrated using International Standard 66/387.*

Storage Instruction

Store the reagents at 2 - 8 ℃ until expiration date.

Materials Required but Not Supplied

- ✓ Precision pipette to deliver 20-100 µL
- ✓ Disposable pipette tips
- ✓ Distilled or deionized water

- ✓ Microplate washer (recommended)
- ✓ Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

Precautions for Use

- LImitation

✓ All the reagents within the kit are calibrated for the direct determination of thyroglobulin in human serum. The kit is not calibrated for the determination of thyroglobulin in saliva, plasma or other specimens of human or animal origin.

✓ Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.

✓ Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.

✓ The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

- Procedural cautions and warnings

Follow the good laboratory practices (GLP) when handling kit reagents:

✓ Do not pipette by mouth.

✓ Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.

✓ Wear protective clothing and disposable gloves when handling the specimens and kit reagents.

✓ Wash hands thoroughly after performing the test.

✓ Avoid contact with eyes; use safety glasses; in case of contact, flush with water immediately and contact a doctor.

✓ Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

✓ Avoid microbial contamination of reagents.

✓ A standard curve must be established for every run.

✓ It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.

✓ The controls (included in kit) should be included in every run and fall within established confidence limits.

✓ When the use of water is specified for dilution or reconstitution, use deionized or distilled water.

✓ All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.

✓ Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.

✓ When reading the microplate, the presence of bubbles in the microwells will affect the optical densities

(ODs). Carefully remove any bubbles before performing the reading step.

- ✓ Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- ✓ The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
- ✓ When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- ✓ To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- ✓ Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
- ✓ Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

- Safety cautions and warnings

All serum samples should be considered a potential biohazard and handled with the appropriate precautions. POTENTIAL BIOHAZARDOUS MATERIAL Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water.

- Limits of the test

- ✓ Highly lipemic, hemolyzed or grossly contaminated specimens should not be used.
- ✓ It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond 10 minutes to avoid assay drift.
- ✓ If more than 1 plate is used, it is recommended to repeat the dose response curve.
- ✓ Do not touch the bottom of the wells.
- ✓ The presence of autoantibodies to TPO is confirmed when the serum level exceeds 30 IU/ml. The clinical significance of the result, coupled with anti-thyroglobulin activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests.
- ✓ About 10 % of asymptomatic specimens may present with anti-TPO autoantibodies reflecting the prevalence in apparently healthy populations. The prevalence of anti-TPO may also depend on age, gender and geographic region of the selected population.

Assay Protocol

Reagent Preparation

Working washing solution: Thoroughly shake washing solution concentrate. To make working washing solution take required amount of concentrate and mix with purified water (1:25 ratio) in a separate vial. Thoroughly mix the solution. Working washing solution may be stored for 3 days at 2-8℃ temperature.

Sample Preparation

Collection of blood samples should be implemented according to the current practices. Serum only may be used. Separate serum as soon as possible to avoid any haemolysis. Extensive haemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in plain redtop venipuncture tube without additives and gel barrier. Samples can be stored at 2-8℃ not more than for 72 hours; they may be deep-frozen at -20℃. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed haemolysis, hyperlipedemia and which were preserved by sodium azide must not be analyzed.

Assay Procedure

All reagents must reach room temperature before use. Standards, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate, add one or two wells for TMB control (blank). Replace any unused microtiter wells back into the aluminum bag, do not remove silica gel drier, and then place the package into plastic bag, seal and store at 2-8℃ for 1 month.
2. Pipette 90 µL of sample diluent 1 to the dilution plate and add 10 µL of the samples to be tested (first serum dilution ratio is 1:10). Carefully mix fluid in wells by gentle pipetting. Blue-violet color should change to blue-green. If you don't observe a change of the color then test result may be false, or there is no serum added to the well.
3. Pipette 100 µL of Calibrators and Control into the assigned wells of the working plate.
4. Pipette 90 µL of sample diluent 2 to the wells of working plate meant for the serum samples and pipette 10 µL of the prediluted sera samples from preliminary plate into these wells (final serum dilution ratio is 1:100). Carefully mix fluid in wells by gentle pipetting.
5. Cover the strips with a plate lid and incubate for 45 min at room temperature on a shaker (approximately 500-800 rpm).
6. Wash the wells 3 times with 300 µL of working washing solution per well and tap the plate firmly against

absorbance paper to ensure that it is dry (the use of a washer is recommended).

7. Add 100 µL of Conjugate to all wells.
8. Cover the strips with a plate lid and incubate for 45 min at room temperature on a shaker (approximately 500-800 rpm).
9. Wash the wells 5 times with 300 µL of working washing solution per well and tap the plate firmly against absorbance paper to ensure that it is dry (the use of a washer is recommended).
10. Pipette 100 µL of TMB substrate into each well at timed intervals.
11. Incubate for 20-30 minutes at room temperature in a dark place
12. Pipette 150 µL of stopping reagent into each wells at the same time intervals as in step 7. Gently mix for 5-10 seconds.
13. Read the plate on microplate reader at 450 nm within 20 minutes after addition of the stopping reagent.

Data Analysis

Calculation of Results

1. Calculate the mean absorbance value of each calibrator duplicate.
2. Draw a calibrator curve on graph paper with the mean absorbance on Y axis and the calibrator concentration on the X axis.
3. Calculate the mean absorbance values for each specimen.
4. Read the values of the unknowns anti-TPO concentration in IU/ml directly off the calibrator curve.

Typical tabulated data (example, do not use for your own calculation):

Calibrator	OD1	OD2	Mean OD-blank	Value (IU/ml)
0	0.048	0.051	0.050	0
1	0.350	0.328	0.339	25
2	1.295	1.222	1.259	100
3	2.087	2.042	2.065	250
4	2.504	2.515	2.510	500
unknown	0.778	0.760	0.769	60

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

The absorbance (OD) of Calibrator 4 should be ≥ 1,3.

Calculated value of Control serum should be within established range.

Performance Characteristics

- Analytical sensitivity

The lower detection limit is 2 IU/ml. The sensitivity was calculated by determining the variability of the 0 IU/ml serum calibrator and using the 2 SD (95% certainty) statistics.

- Specificity (cross reactivity)

Interferences from ANA, DNA, thyroglobulin and rheumatoid antibodies were found to be insignificant.

- Intra-assay precision

Three samplies were assayed 15 times each on the same calibrator curve. The results (in IU/ml) are tabulated below:

Sample	Mean	SD	CV %
1	365.0	25.2	6.9
2	213.8	15.3	7.2
3	123.0	8.5	6.9

- Inter-Assay precision

Three samplies were assayed 3 times. The results (in IU/ml) are tabulated below:

Sample	Mean	SD	CV %
1	345.7	19.9	5.8
2	201.3	15.2	7.5
3	119.1	8.7	7.3

- Expected normal Value

A normal range of less 30 IU/ml anti-TPO was obtained by testing serum specimens from 250 individuals determined as normal by Thyroid-T4 free and Thyroid-TSH assays. It is strongly recommended that each laboratory should determine its own normal range values.

- Accuracy

This Anti-TPO-ELISA was compared with a reference Chemiluminescent microparticle immunoassay. The total number of specimens was 135 (0-974 IU/ml). The least square regression equation and correlation coefficient were computed for this ELISA in comparison with the reference method. The least square regression equation is y= 0.92(x) + 10.2 with correlation coefficient 0.95.

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								