

## Product Information

### Thyroid Peroxidase IgG ELISA kit

Catalog Number: EA100926

Storage Temperature: 2 – 8°C

## Instruction for Use

### Intended Use

The Thyroid Peroxidase (TPO) IgG ELISA Kit is intended for the detection of IgG antibody to Thyroid Peroxidase in human serum or plasma.

### Background

Thyroid peroxidase (TPO) is the major autoantigen (933 amino acid residue) in the thyroid microsomal antigen (TMA) particle. The purification and preparation of this antigen has made testing for TMA antibodies obsolete. Assays for TPO antibodies include ELISA, precipitation of radiolabeled TPO-bound autoantibodies with protein A, competition for TPO binding to immobilized anti-TPO murine monoclonal antibodies, autoantibody capture by TPO-coated beads and chemiluminescence. All tests correlate well with detection of TMA. ELISA using TPO recombinant antigen is the most popular assay. Detection of TPO antibodies is strong evidence against a goiter or non-autoimmune causes of hypothyroidism. The annual risk for the development of hypothyroidism is 3% to 4% per year if TPO antibodies are present and TSH is elevated. TPO antibodies are present in 8-9% normal controls, 57-74% patients with Graves disease, 99- 100% of Hashimoto disease or idiopathic myxedema, 19% with differentiated thyroid cancer, no patients with subacute thyroiditis and 11% of those with other miscellaneous non-autoimmune thyroid diseases. The prevalence of positive TPO antibodies is higher in elderly (mean age 80 years) women (10%) compared to elderly men (2%). Autoantibody concentration in centenarians also decreases. Studies of TPO epitopes in each domain, A and B, and detection of their specific autoantibodies suggest that the epitope-specific TPO antibodies ratio (A/B) does not change over time in individual patients and that TPO epitope autoantibody patterns may be inherited.

### Principle of the Test

Diluted patient serum is added to wells coated with purified TPO recombinant antigen. TPO IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

## Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with recombinant TPO antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Enzyme conjugate: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12 ml
5. Calibrator: 1 Vial (ready to use)	1 ml
6. Positive Control: 1 vial (ready to use)	1 ml
7. Negative Control: 1 vial (ready to use)	1 ml
8. Stop Solution: 1 bottle (ready to use)	12 ml
9. Wash concentrate 20X: 1 bottle	25 ml

### Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

### Disclaimer

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

### Storage and Stability

1. Store the kit at 2-8 °C
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

### Specimen Collection and Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

### Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

### Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate

- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities
1. Place the desired number of coated strips into the holder.
  2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10  $\mu$ l of the sample to 200  $\mu$ l of sample diluent. Mix well.
  3. Dispense 100  $\mu$ l of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100  $\mu$ l sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
  4. Remove liquid from all wells. Wash wells three times with 300  $\mu$ l of 1X wash buffer. Blot on absorbance paper or paper towel.
  5. Dispense 100  $\mu$ l of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
  6. Remove enzyme conjugate from all wells. Wash wells three times with 300  $\mu$ l of 1X wash buffer. Blot on absorbance paper or paper towel
  7. Dispense 100  $\mu$ l of TMB substrate and incubate for 10 minutes at room temperature.
  8. Add 100  $\mu$ l of stop solution.
  9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

### Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

### Quality Control

The test run may be considered valid provided the following criteria are met:

1. If the O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should fall within the range specified on the COA/label.

### Limitations of the Test

1. Lipemic or hemolyzed samples may cause erroneous results.

### References

1. Fan JL; Patibandla SA; Kimura S; Rao TN; Desai RK; Seetharamaiah GS; Kurosky A; Prabhakar. BS Purification and characterization of a recombinant human thyroid peroxidase expressed in insect cells. *J Autoimmun* 1996; 9(4):529-36.
2. Feldt-Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. *Clin Chem* 1996;42:160-3.
3. Franke WG; Schimming C; Wunderlich G. Can thyroid peroxidase be used as a complementary tumor marker besides thyroglobulin? Preliminary experience with determination of TPO in differentiated thyroid carcinomas. *Anticancer Res* 1997;17(4B):2999-3002.
4. Haapala AM; Hyöty H; Parkkonen P; Mustonen J; Soppi E. Antibody reactivity against thyroid peroxidase and myeloperoxidase in autoimmune thyroiditis and systemic vasculitis. *Scand J Immunol* 1997; 46(1):78-85.

5. Mariotti S, Caturegli P, Piccolo P, Barbesino G, Pinchera A. Antithyroid peroxidase autoantibodies in thyroid diseases. *J Clin Endocrinol Metab* 1990;71:661-9.
6. Massart C, Guilhem I, Gibassier J, Allannire H, Nicol M. Comparison of thyroperoxidase and microsomal antibody assays in sera from patients with Graves disease. *Clin Chem* 1991;37:1777-80.
7. Nakamura H; Genma R; Mikami T; Kitahara A; Natsume H; Andoh S; Nagasawa S; Nishiyama K; Chida K; Sato A; Yoshimi T. High incidence of positive autoantibodies against thyroid peroxidase and thyroglobulin in patients with sarcoidosis. *Clin Endocrinol (Oxf)* 1997; 46(4):467-72.
8. Roti E, Gardini E, Minelli R, Bianconi L, Braverman LE. Prevalence of anti-thyroid peroxidase antibodies in serum in the elderly: comparison with other tests for anti-thyroid antibodies. *Clin Chem* 1992;38:88-92.

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