



Thyroid Peroxidase [TPO] IgG (human) ELISA Kit

7/14

(Catalog # K5351-100, 100 assays; Store at 2-8°C)

I. Introduction:

Thyroid peroxidase (TPO) is the major autoantigen (933 amino acid residue) in the thyroid microsomal antigen (TMA) particle. 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, 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. In BioVision's TPO IgG ELISA Kit, sample is added to the 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.

II. Application:

Detection of IgG Ab to TPO

III. Specificity:

Human

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K5351-100	Part No.
Plate coated with TPO recombinant Ag	12 stripsx8 wells	K5351-100-1
Sample Diluent	22 ml	K5351-100-2
Calibrator	1 ml	K5351-100-3
Positive Control	1 ml	K5351-100-4
Negative Control	1 ml	K5351-100-5
Enzyme Conjugate	12 ml	K5351-100-6
Wash Concentrate (20X)	25 ml	K5351-100-7
TMB Substrate	12 ml	K5351-100-8
Stop Solution	12 ml	K5351-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Concentrate:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

VIII. Warning & Precautions:

- Potential biohazardous materials: The Calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Do not pipette by mouth.
- 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.
- Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
- This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for up to 7 days or store frozen at (-20°C) for up to six months. Avoid multiple freeze-thaw cycles. Lipemic or hemolyzed samples may cause erroneous results.

X. Assay Protocol:

Bring all specimens and kit reagents to room temperature and gently mix. 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.



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 µl of the sample to 200 µl of sample diluent. Mix well.
3. Pipet 100 µl of diluted sera, calibrator and controls into designated wells. For the reagent blank, dispense 100 µl sample diluent into designated well. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 min. at room temperature.
4. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
5. Add 100 µl of enzyme conjugate to all wells & incubate for 20 min. at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 min. at room temperature.
8. Add 100 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stop solution. A dual wavelength is recommended with reference filter of 600-650 nm.

XI. Calculation: Calculate cut-off value: Calibrator OD x Calibrator Factor (CF). Calculate the Ab (Antibody) Index of each determination by dividing the mean values of each sample by cut-off value.

Example of typical results:

Calibrator mean OD = 0.8
 Calibrator Factor (CF) = 0.5
 Cut-off Value = 0.8 x 0.5 = 0.400
 Positive control O.D. = 1.2
 Ab Index = 1.2 / 0.4 = 3
 Patient sample O.D. = 1.6
 Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met: 1. 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 be greater than 1.2.

INTERPRETATION

The following is intended as a guide to interpretation of TPO antibody test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

1. Antibody Index Interpretation: <0.9 No detectable TPO antibody by ELISA, 0.9 -1.1 Borderline positive. Follow-up testing is recommended if clinically indicated, >1.1 detectable TPO antibody by ELISA.

2. Converting of Ab Index to IU/mL: As an option, TPO Ab index may be converted to IU/mL by multiplying Ab index value by 50.

International units may then be interpreted as follows:

<50 IU/mL: Negative
 50-75 IU/mL: Borderline positive
 > 75 IU/mL: Positive

Precision

Intra-Assay

Sample	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation (%)
1	16	1.75	0.1	5.7
2	16	0.92	0.07	7.6
3	16	0.19	0.02	10.5

Inter-Assay

Sample	No. of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	10	2.12	0.17	8.0
2	10	1.05	0.09	8.6
3	10	0.21	0.03	14.2

Sensitivity and specificity: 134 patient sera were tested by this ELISA and a reference ELISA method. 39 were positive and 92 were negative by both methods (98% agreement). The results are summarized below:

	TPO IgG ELISA		
	+	-	Total
Reference ELISA Kit	39	1	40
	2	92	94
Total	41	93	134

XII. RELATED PRODUCTS:

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|---|---|
| Thyroxine (T4) (human) ELISA Kit (K7413) | Thyroxine (T4) (mouse/rat) ELISA Kit (K7421) |
| Triiodothyronine (T3) (human) ELISA Kit (K7423) | Triiodothyronine (T3) (mouse/rat) ELISA Kit (K7422) |
| TPO (mouse) ELISA Kit (K4753) | TPO Antibody (5351R, 5352) |
| TPO, human recombinant (4351) | TPO, murine recombinant (4352) |
| TPO, rat recombinant (7221) | Human CellExp™ TPO, human recombinant (6483) |

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