Product Information

Thyroid Peroxidase IgG ELISA

Catalog Number SE120124
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
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 3–4% 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 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.

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

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with TPO recombinant Ag</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle (ready to use)</td>
<td>22 mL</td>
</tr>
<tr>
<td>Calibrator: 1 Vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Positive Control: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Wash concentrate 20x: 1 bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

Reagents and Equipment Required but Not Provided.
1. Distilled or deionized water
2. Precision pipettes. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Absorbent paper or paper towel

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.
Preparation Instructions
Sample 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 of serum sample.

20x Wash Buffer 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).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab Index for Positive control should be >1.2.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 21-fold dilution of test samples, by adding 10 μL of the sample to 200 μL of Sample Diluent. Mix well.
3. Dispense 100 μL of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 μL of 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 μL of 1x wash buffer. Blot on absorbent paper or paper towel.
5. Dispense 100 μL 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 μL of 1x wash buffer. Blot on absorbent paper or paper towel.
7. Dispense 100 μL of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 μL of Stop Solution.
9. Read O.D. at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.
Results
Calculations

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure the value is checked on every kit.

2. Calculate cut-off value: Calibrator OD x Calibrator Factor (CF).

3. 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

Note: Lipemic or hemolyzed samples may cause erroneous results.

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.

Antibody Index Interpretation

<0.9 – No detectable TPO antibody by ELISA
0.9–1.1 – Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1 – Detectable TPO antibody by ELISA

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

References


RGC,CH,MAM 10/14-1