Thyroglobulin (TG) Antibody ELISA

Catalog Number SE120123
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Thyroglobulin is a water soluble glycoprotein that is involved in the storage and synthesis of thyroid hormones. The thyroid microsomal antigen has been shown to be the enzyme thyroid peroxidase (TPO). Antibodies to thyroglobulin and/or microsomal antigen are present in most patients with goitrous thyroiditis (Hashimoto disease), atrophic thyroiditis, and 70–90% of Graves’ disease. Antibodies are also found in about half of the patients with primary hypothyroidism and thyrotoxicosis, and 10–20% of patients with simple goiters and thyroid tumors. There is also a relationship between thyroid antibodies and diabetes mellitus. Thyroid autoantibodies are present in 6–7% of normals and their incidence increases with age. Classically, autoantibodies to thyroid antigens are detected by precipitation reactions, hemagglutination, and by immunofluorescence. However, the tests are subjective and lack high sensitivity. Enzyme-Linked Immunosorbent Assays (ELISAs) combine greater sensitivity, objective reading, and ease of use.

The Thyroglobulin (TG) Antibody ELISA kit is intended for the detection of IgG antibody to Thyroglobulin (TG) in human serum or plasma. Diluted serum is added to wells coated with purified TG recombinant antigen. TG 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 TG 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 TG antigen</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
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

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.

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 TG antibody test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

**Antibody (Ab) Index Interpretation**

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

**Converting of Ab Index to IU/mL**

As an option, TG Ab index may be converted to IU/mL by multiplying Ab index value by 100. International units may then be interpreted as follows:

<100 IU/mL: Negative  
100-150 IU/mL: Borderline positive  
>150 IU/mL: Positive
References


