

Thyroglobulin Enzyme Immunoassay

Numerous thyroid disorders are characterized by an autoimmune response^{1,2} which can be both humoral and cell mediated. Detection of clinically significant levels of thyroid antibodies is used for the diagnosis of thyroid diseases and thyroid-related abnormalities such as: thyroid related at-risk pregnancy³, chronic lymphocytic thyroiditis (Hashimoto's disease), Grave's disease, non-toxic goiter, myxedema, thyroid cancer, hyperthyroidism, and subacute thyroiditis. In addition, significant titers of anti-thyroid antibodies have been reported in patients with Addison's disease, myasthenia gravis, liver disease, diabetes mellitus and various connective tissue diseases.

Thyroglobulin autoantibodies are found in the sera of most autoimmune thyroid disease patients; but until now, thyroid autoimmunity testing has not been considered during comprehensive thyroid diagnostic work-ups due to the inaccuracy of available methodologies.

The **ImmunoWELL** Thyroglobulin Antibody Test utilizes purified human thyroglobulin in an EIA format. The ImmunoWELL EIA Test uses a multipoint (five) calibration curve to generate accurate, quantitative results which can be reported in standardized International Units (IU).

Expected Results

Moderate to high levels of anti-TPO may indicate a variety of thyroid diseases and abnormalities. Asymptomatic subjects may have low anti-TPO levels², but the significance of these low level reactions has not been established. Absorbance values can be referenced to IU/mL interpretive ranges (66/387 WHO standard)

Performance Characteristics

Passive hemagglutination positive samples were evaluated to test sensitivity. This test classifies titers less than 1:160 as low and greater than or equal to 1:640 as moderate to high reactivity. Based on this classification, the **ImmunoWELL** Thyroglobulin Test is 100% sensitive. The EIA method also allows further discrimination of specific low level reactivity of anti-thyroglobulin reactions. The **ImmunoWELL** Thyroglobulin Test is 97% specific.

Ordering Information

Product Description	Quantity	GenBio Product No.
ImmunoWELL Thyroglobulin Antibody Test	1 kit / 96 wells	3030
<i>Also available from GenBio</i>		
ImmunoWELL Microsome (TPO) Antibody Test	1 kit/96 wells	3020

Principle

The **ImmunoWELL** Test utilizes an EIA microtiter plate technique for the detection of antibodies. Serum is added to antigen coated microtiter wells and allowed to react. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman IgG antibodies are allowed to react with bound antibodies. The bound peroxidase reacts with tetramethylbenzidine (TMB), the chromogenic substrate, developing a color. Finally, the substrate reaction is stopped and the optical density is read with a microwell spectrophotometer.

Procedural Summary

1. Prepare Wash Buffer from Wash Buffer Concentrate
2. Dilute each control and specimen 1:100 in Specimen Diluent
3. Add 100 μ L of Specimen Diluent into the first well as a substrate blank.
4. Add 100 μ L of prediluted Calibrators, diluted Controls and Specimens to coated microwells and incubate 30 min at room temperature
5. Aspirate wells and wash microwells three times with Wash Buffer
6. Add 100 μ L of Conjugate to wells and incubate 30 minutes at room temperature
7. Aspirate microwells and wash wells three times with Wash Buffer
8. Add 100 μ L of Substrate to wells and incubate 30 minutes at room temperature
9. Add 100 μ L Stop Solution to wells and read results at 450nm

References

1. Czarnocka, B, J Ruf, M Ferrand, P Carayon, and S Lissitzky. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. FEBS 190(1):147-152 (1985)
2. Volpe, R. The role of autoimmunity in hypoendocrine and hyperendocrine function. Ann Int Med 87:86-99 (1977)
3. Stagnoro-Green, A, SH Roman, RH Cobin, E El-Harazy, M Alvarez-Marfany, TF Davies. Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies. JAMA 264(11):1422-1425 (1990)

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