Anti-TPO is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against thyroid peroxidase in human serum or plasma. Samples are typically used at 1:100 or more in the ELISA test (115 min, at room temp). Isotype-specific ELISA kits measures only one isotype (IgA, IgG or IgM). ELISA kits for mouse, rat, and human samples are currently available but other species can be requested as special order. All ELISAs follow the similar design so this brief brochure represents general features of anti-TPO ELISAs. Detailed manual is provided with the kit. This kit does not measure other Ig’s subclasses such as IgM, IgA or IgE.

### Human Anti-TPO ELISA Kit Features

- Highly specific TPO pre-coated, stabilized, ready-to-use 96-well strip plate, suitable for multiple runs over 6-12 months.
- Human anti-TPO standards (0, 33, 100, 330,1000, 3000 IU/ml).
- Anti-TPO negative and Positive controls
- 100 ul samples (1:100 diluted); 1 hr assay (30+15+15 incubation steps at room temp.)
- Sensitivity ~ 5 IU/ml
- Specific for autoantibodies directed to TPO
- Storage (2-4°C), Shelf life : 12-18 months
- Contains all necessary reagents. For in vitro research use only.

### Assay Procedure:

**Allow all reagents to reach room temperature. Arrange and label required number of strips.**

**Step 1.** Pipet 100 ul each of pre-diluted stds, and controls. Incubate for 30 mins at RT.

**Step 2.** Aspirate and wash 3X. Add 100 ul of Conjugate to all wells and incubate at RT for 15 mins.

**Step 3.** Decant and wash 4X. Add 100 ul TMB substrate and incubate for 15 mins at RT.

**Step 4.** Add 100 ul stop solution to all wells, and incubate for 5 mins at RT.

**Step 5.** Measure OD at 450 nm.

### Performance Characteristics

**Calibration:**
The quantitative assay system is calibrated against the WHO reference preparation MRC 66/387 for Anti-TPO antibodies. WHO MRC 66/387 is determined as 1000 IU/ml Anti-TPO.

**Results:** (Anti-TPO (IU/ml))
- Normal: < 50
- Borderline: 50 – 75
- Elevated: > 75

**Specificity:** The microplate is coated with antigen, isolated from human thyroid tissue and highly purified by affinity chromatography. The Anti-TPO is a specific test kit for the quantitation of IgG-class autoantibodies directed against human thyroid peroxidase (TPO). No cross reactivity to the other ENA-antibodies have been observed.

**Precision:** Intra-assay (3.1-9.7%), Inter-assay (1.6-2.9%).

### General Information

Thyroid disorders are the most prevalent of all autoimmune diseases. Thyroid autoimmune diseases are associated with the occurrence of differentiated autoantibodies and are thought to be related to a genetic pre-disposition. These autoantibodies are directed against membrane-located and/or extracellular antigens of the thyroid cells. - Thyroglobulin (hTg), a water soluble glycoprotein with a molecular weight of approx. 660,000 Dalton, is the principal constituent of the thyroid colloid sharing about 75% of its mass. - Synthesis of the thyroid hormones T3 and T4 is based on the oxidative iodination of thyroxine residues of the thyroglobulin molecule. Within the cell thyroglobulin is trans-ported by the microsomes. Together with the secretion of T3 and T4 also small amounts of hTg are liberated into circulation. - The microsomal antigen of the thyroid is an integral membrane protein of the microsomes. It has been characterized as the enzyme Thyroid Peroxidase (TPO) with a molecular weight of nearly 110,000 Dalton. - The TSH-Receptor is a regulatory protein embedded into the thyroid cell membrane effecting synthesis and release of the thyroid hormones as well as cellular growth. - The so-called Colloid-Antigen 2 CA2. Besides these antibodies to functional antigens, antibodies directed against the circulating thyroid hormones T3 and T4 may occur. In Graves’ Diseases, an immunogenetic form of hyperthyroidism often additional antibodies occur, which are directed against eye muscle antigens. They cause the endocrine ophalmopathy. Autoantibodies are found in inflammatory diseases as well as in thyroid autoimmune disorders. Various symptoms of thyroid diseases, like goiter, thyroid pain, hyperthyroidism and hypothyroidism may be caused by immunogenetic factors and the occurrence of organ specific antibodies. This underlines the clinical relevance for autoantibody determination for the assessment of thyroid diseases. Most important autoimmune diseases of the thyroid gland are: Hashimoto’s Thyroiditis Primary Myxedema Graves’ disease (often associated with endocrine ophalmopathy) and other asymptomatic, for example postpartum thyroiditis. The occurrence of Anti-TG and Anti-TPO autoantibodies at the same time seem to be related to their functional association. Thyroid-sine amino acid residues of the thyroglobulin molecule, as primary protein for the synthesis of the thyroid hormones T3 and T4, are actively iodinated in association with the thyroid peroxidase (TPO). TSH acts stimulating synthesis and release of thyroid hormones in close cooperation of all the proteins. This makes the simultaneous appearance of all these antibodies plausible. Persisting inhibition of the peroxidase activity by specific autoantibodies (Anti-TPO Ab’s) causes a decrease in the synthesis of thyroid hormones and thus hypothyroidism. Especially at the end of pregnancy, determination of thyroid antibodies may be a helpful diagnostic tool in the early diagnosis of an onset of post-partum hypothyroidism (Hashimoto’s post partum depression). Hashimoto diseases are often associated with highly elevated titers of thyroid autoantibodies. The concentration of antibodies against thyroglobulin exceeds the titer of Anti-TPO antibodies, whereas 3 in Graves’ disease the opposite situation is found, with a stronger elevation of the Anti-TPO antibodies. Additionally, also high concentrations of TSH receptor antibodies are characteristic for both diseases. The following table summarizes the distribution of autoantibodies in thyroid autoimmune diseases.

**Note:** Detailed manual will be supplied with the kit.

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**Human Anti-thyroid peroxidase (TPO) IgG ELISA Kit Cat #3300-350-TPG**

**A Typical Std. Curve**