Instruction Manual

TPO (Thyroid Peroxidase) IgG ELISA

Enzyme immunoassay based on microtiter plate
for the detection and quantitative determination
of human IgG antibodies against **TPO**
in serum and plasma

CE

Cat. No.: ILE-TPG01
Storage: 4-8°C
For in-vitro diagnostic use only

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IMMUNOLAB GmbH, Otto-Hahn-Str. 16, D-34123 Kassel
Tel: +561 491742-0, Fax: +561 491742-20, E-Mail: info@immunolab.de

Contents **Page**

1. Intended Use

The Immunolab TPO IgG Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG antibodies against TPO in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Immunolab.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

2. General Information

In various diseases of the thyroid, autoantibodies against thyroid antigens are found in the serum. The most important antigens are:
- Thyroglobulin (TG)
- Thyroid peroxidase (TPO) (formerly known as microsomal antigen)
- TSH-Receptor

The determination of these autoantibodies has a diagnostic and a predictive value. Women express thyroid autoimmunity more frequently than men. This tendency is even more obvious at the postmenopausal period. These women with significant autoantibody titers against thyroid microsomal antigen or thyroid peroxidase and against thyroglobulin are prone to develop chronic thyroiditis resulting in thyroid atrophy and hypothyroidism. It is important to screen all mothers-to-be for thyroid autoimmunity and to determine carefully the titers of thyroid autoantibodies and thyroid function in the postpartum period. The so-called idiopathic myxoedema is now well recognized as the end-stage of an autoimmune, atrophic chronic thyroiditis, with its serological signs, the anti-TG and anti-TPO autoantibodies. In younger patients, the combination of a firm goiter with high titers of anti-TG and anti-TPO autoantibodies is highly suggestive of Hashimito's disease, a chronic lymphomonocytic thyroiditis. In Graves Basedow's disease, significant anti-TG and anti-TPO autoantibody titers indicate the coexistence of chronic thyroiditis in association with the toxic goiter. Indeed, hyperthyroid patients with high titers of anti-TG and anti-TPO autoantibodies exhibit a greater susceptibility to develop hypothyroidism in the long term after such treatments. The determination of anti-TSH receptor autoantibody titer is also very important in the evaluation of a patient suffering from Graves Basedow's disease. In general practice, the detection of thyroid autoimmunity may signify that the patient is at risk of other autoimmune diseases including pernicious anaemia, Sjögren's and Sicca Syndromes, adrenal insufficiency and hypoparathyroidism.

3. Principle of the Test

The Immunolab TPO IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). TPO antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use calibrators are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized TPO antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

4. Limitations, Precautions and General Comments

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, and they should not be mixed with one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. Reagents Provided

Store kit components at 2-8^{o}C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25^{o}C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8^{o}C. The opened kit should be used within three months.

Components	Volume / Qty.
TPO antigen coated microtiter strips	12
Calibrators with 0, 30, 100, 300, 1000 and 3000 IU/mL	6 x 2 mL
Positive Control	2 mL
Negative Control	2 mL
Enzyme Conjugate	15 mL
Substrate	15 mL
Stop Solution	15 mL
Sample Diluent	60 mL
Washing Buffer (10×)	60 mL
Plastic foils	2
Instruction Manual	1

5.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with a TPO antigen. Ready-to-use.

5.2. Calibrators (Standards)
6 x 2 mL, human serum diluted with PBS, with 0, 30, 100, 300, 1000 and 3000 IU/mL of IgG antibodies against TPO. Addition of 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane. Ready-to-use.

5.3. Positive Control
2 mL, ready to use. The concentration range is given on the vial label.

5.4. Negative Control
2 mL, ready to use. The concentration range is given on the vial label.

5.5. Enzyme Conjugate
15 mL, anti-human-IgG-HRP, in protein-containing buffer solution. Ready-to-use.

5.6. Substrate
15 mL, TMB (tetramethylbenzidin). Ready-to-use.

5.7. Stop Solution
15 mL, 0.5 M sulfuric acid. Ready-to-use.

5.8. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.1 % sodium azide. Ready-to-use.

5.9. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.10. Plastic Foils
2 pieces to cover the microtiter strips during the incubation.

5.11. Plastic Bag
Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Bidistilled water

7. Specimen Collection and Handling

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (4-8°C) for up to 48 hours, for a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the calibrators) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µl serum + 500 µl sample diluent).

8. Assay Procedure

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Calibrators and samples should be assayed in duplicates.
- A calibration curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 4-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the calibrators, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** calibrators and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

Example

	Mean OD Value
Calibrator 1 (0 IU/mL)	0.016
Calibrator 2 (30 IU/mL)	0.319
Calibrator 3 (100 IU/mL)	1.002
Calibrator 4 (300 IU/mL)	1.465
Calibrator 5 (1000 IU/mL)	1.875
Calibrator 6 (3000 IU/mL)	2.222

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

9.1. Quantitative Evaluation

The ready-to-use calibrators of the TPO antibody kit are defined and expressed in International Units (IU/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for the calibrators in International Units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the calibrators are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

The dilution factor of the samples (1:101) has already been reconsidered in the concentration given for the calibrators.

9.2. Interpretation

It is recommended that each laboratory establishes its own range of normal values. All participants in the normal range study were apparently healthy subjects. The normal value range is assumed to be as 95%-percentile.

> 100 IU/mL	positive
60 – 100 IU/mL	borderline
< 60 IU/mL	negative/normal

10. Assay Characteristics

TPO ELISA	IgG
Intra-Assay-Precision	7.3 %
Inter-Assay-Precision	5.2 %
Inter-Lot-Precision	2.9 – 6.3 %
Analytical Sensitivity	2.9 IU/mL
Recovery	99 – 104 %
Linearity	77 – 118 %
Cross-Reactivity	No cross-reactivity to Thyroglobulin
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
Clinical Specificity	100 %
Clinical Sensitivity	100 %

11. References

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