# Anti-TPO

**Intended use**
Immunoassay for the in vitro quantitative determination of antibodies to thyroid peroxidase in human serum. The anti-TPO determination is used as an aid in the diagnosis of autoimmune thyroid diseases.

**Summary**
1-6 Thyroid-specific peroxidase (TPO) is present on the microsomes of thyrocytes and is expressed at its apical cell surface. In synergy with thyroglobulin (Tg) this enzyme has an essential function in the iodination of L-tyrosine and the chemical coupling of the resulting mono- and di-iodotyrosine to form the thyroid hormones T4, T3, and fT3. TPO is a potential autoantigen. Elevated serum titers of antibodies to TPO are found in several forms of thyroiditis caused by autoimmunity. The still frequently found term "microsomal antibody" originates from the time when TPO had not yet been identified as an antigen in autoimmunity caused by microsomes. In the clinical sense the two terms anti- TPO and microsomal antibody can be used synonymously; there are differences, however, with regard to the test methods.
High anti-TPO titers are found in up to 90 % of patients with chronic Hashimoto's thyroiditis. In Graves' disease, 70 % of the patients have an elevated titer. Although the sensitivity of the procedure can be increased by simultaneously determining other thyroid antibodies (anti-Tg, TSH-receptor-antibody - TRAb), a negative finding does not rule out the possibility of an autoimmune disease. The magnitude of the antibody titer does not correlate with the clinical activity of the disease. Initially elevated titers can become negative after lengthy periods of illness or during remission. If antibodies reappear following remission, then a relapse is probable.
Whereas the usual microsomal antibody tests employ unpurified microsomes as an antigen preparation, the anti-TPO tests use a purified peroxidase. The two procedures are of comparable performance in terms of clinical sensitivity, but better lot-to-lot consistency and higher clinical specificity can be expected from anti-TPO tests due to the higher quality of the antigen used. The Enzyme linked immunosorbent assays uses human antigen and rabbit anti-human IgG antibodies (anti-IgG).

**Test principle**
Indirect method, total duration of assay: 70 minutes.
The Anti-TPO ELISA employs solid phase, indirect ELISA method for detection of antibodies to TPO in two-step incubation procedure. Polystyrene microwell strips are pre-coated with highly immunoreactive human TPO antigens. During the first incubation step, anti-TPO specific antibodies, if present, will be bound to the solid phase pre-coated TPO antigens.
The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody(IgG) complexes previously formed and the unbound HRP- conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid.
The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively.

**Reagents**
**Materials provided**
• Coated Microplate, 8 x 12 strips, 96 wells. Pre-coated with human TPO antigen.
• Calibrators, 6 vials, 1 mL each, ready to use; Concentrations: 0(A), 25(B) ,50(C), 100(D), 250(E) and 500(F) IU/mL.
• Enzyme Conjugate, 1 vial, 11.0 mL of HRP (horseradish peroxidase) labeled rabbit anti- human IgG antibodies (anti-IgG) in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
• Serum Diluent: 1 vial, 11mL. Containing buffer salts and a dye
• Wash Solution Concentrate, 1 vial, 25 ml (40X concentrated), PBS-Tween wash solution.
• Substrate, 1 vial, 11ml, ready to use, (tetramethylbenzidine) TMB.
• Stop Solution, 1 vial, 6.0 ml of 1 mol/l sulfuric acid.
• IFU, 1 copy.
• Plate Lid: 2 pieces.

**Materials required (but not provided)**
• Microplate reader with 450nm and 620nm wavelength absorbent capability.
• Microplate washer.
• Incubator.
• Plate shaker.
• Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
• Absorbent paper.
• Distilled water

**Precautions and warnings**
• For in vitro diagnostic use only. For professional use only.
• Make sure that all reagents are within the validity indicated on the kit box and of the same lot.Never use reagents beyond their expiry date stated on labels or boxes.
• Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
• Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
• Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
• Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
• Do not use reagents beyond the labeled expiry date.
• Do not mix or use components from kits with different batch codes.
• If more than one plate is used, it is recommended to repeat the calibration curve.
• It is important that the time of reaction in each well is held constant to achieve reproducible results.
• Ensure that the bottom of the plate is clean and dry.
• Ensure that no bubbles are present on the surface of the liquid before reading the plate.
• The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

**Storage**
• Store at 2-8℃.
• Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
• Seal and return unused calibrators to 2-8 °C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles.
• Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

**Specimen collection and preparation**
• Collect serum samples in accordance with correct medical practices.
• Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 3 days at 2-8 °C, and 1 month at -20 °C. Recovery within 90-110 % of serum value or slope 0.9-1.1. Freeze only once.
• The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
• Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
• Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
• Avoid grossly hemolytic, lipemic or turbid samples.
• Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
• If proper sample collection and preparation cannot be verified, or if samples have been disru ed due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
• Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
• Adjust the incubator to 37 °C.
• Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
• Don't use Substrate if it looks blue.
• Don't use reagents that are contaminated or have bacteria growth.

**Quality control**
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

**Test procedure**
• Use only the number of wells required and format the microplate wells for each calibrator and sample to be assayed.
• Add 100 µL of calibrators to each well.
• Add 100 µL of Sample Diluent (Green color) to each well Except the Calibrator-wells.
• Add 5 µL of Sample to each Sample Diluent well (NOTE: Reagents in Wells will turn Blue color from Green), then shake 30 seconds.
• Cover the plate with a plate lid and incubate at 37 °C for 30 minutes.
• Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
• Add 350 µL of wash solution, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
• Add 100 µL of enzyme conjugate,

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• Cover the plate with a plate lid and incubate at 37 °C for 30 minutes.
• Add 350 µL of wash solution, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
• Add 100 µL of Substrate to each well. Cover and incubate at ambient temperature (18-25°C) in the dark for reaction for 10 minutes. Do not shake the plate after substate addition.
• Add 50 µL of stop solution to each well.
• Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
• Read the absorbance of each well at 450 nm ( using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

**Calculation**
• Record the absorbance obtained from the printout of the microplate reader.
• Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
• Plot the common logarithm of absorbance against concentration in IU/mL for each calibrator.
• Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay

| Sample | Value (IU/mL) | Absorbance |
|---|---|---|
| **Calibrator A** | 0 | 0.011 |
| **Calibrator B** | **25** | **0.241** |
| **Calibrator C** | **50** | **0.528** |
| **Calibrator D** | **100** | **1.129** |
| **Calibrator E** | **250** | **1.974** |
| **Calibrator F** | **500** | **2.868** |
| **Control 1** | **26.57** | **0.259** |
| **Control 2** | **277.96** | **2.074** |
| **Sample** | **52.41** | **0.557** |

**Limitations – interference**
• The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb <0.93 mmol/L or < 1.5 g/dL), lipemialipemia (triglycerides < 23.9 mmol/L or < 2100 mg/dL) and biotin (< 40.9 nmol/L or < 10 ng/mL).
• Criterion: Recovery within ± 15 % of initial value.
• Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
• No interference was observed from rheumatoid factors up to a concentration of 1500 IU/ mL.
• In vitro tests were performed on 23 commonly used pharmaceuticals. No interference with the assay was found.
• In rare cases, interference due to extremely high titers of antibodies to analytespecific antibodies can occur. These effects are minimized by suitable test design.
• For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**Limits and ranges**
**Measuring range**
5.0-500 IU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 5.0 IU/mL. Values above the measuring range are reported as > 500 IU/mL.
Lower limits of measurement
Lower detection limit
Lower detection limit:< 5.0 IU/mL
The lower detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

**Expected values**
Studies conducted with the Anti-TPO assay covering a total of 217 healthy subjects confirmed the currently used threshold value of 34 IU/mL; this value corresponds to the 94th percentile. Therefore, values in excess of 34 IU/mL are considered positive for the presence of anti-TPO autoantibodies.
We have not studied the reference intervals in children, adolescents and pregnant women. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

**Specific performance data**
Representative performance data are given below. Results obtained in individual laboratories may differ.

**Precision**
Precision was determined using reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

| | | Repeatability* | | Intermediate precision | |
|---|---|---|---|---|---|
| Sample | Mean | SD | CV | SD | CV |
| | | IU/mL | % | IU/mL | % |
| Human Serum 1 | 15.82 | 0.81 | 5.17 | 1.24 | 7.85 |
| Human Serum 2 | 134.27 | 5.76 | 4.29 | 8.40 | 6.26 |
| Human Serum 3 | 209.91 | 7.82 | 3.78 | 11.27 | 5.45 |
| PC Universal 1 | 33.84 | 1.87 | 5.53 | 2.15 | 6.38 |
| PC Universal 2 | 258.43 | 10.00 | 3.87 | 11.81 | 4.57 |

*Repeatability = within-run precision

**Method comparison**
A comparison of the anti-TPO assay (y) with the Beckman Coulter Access Anti-TPO (x) using clinical samples gave the following correlations: Number of samples measured: 112

**Linear regression**
y = 1.0254x + 0.0193
r = 0.9731
The sample concentrations were between approx. 5.0 and 420 IU/mL.

**Analytical specificity**
No influence with human autoantibodies to thyroglobulin (< 390 IU/mL) was detectable.

**References**

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PRESENTACION:
CONT: 96 TEST
CODIGO:RSET112-3