



# Anti-TPO



## Enzyme-immunoassay for the quantitative determination of anti-Thyroid Peroxidase antibodies in serum or plasma

**REF** GD7320 00

**IVD**

### INDICATION

Anti-TPO antibodies (formerly - thyroid microsomal antibodies) are directed against a target protein - thyroid peroxidase (TPO) - located in the smooth endoplasmic reticulum of thyroid cells. The presence of anti-TPO antibodies in serum is associated with thyroid autoimmune diseases (Graves' disease and Hashimoto's thyroiditis). Anti-TPO antibodies mostly belong to the IgG class. Low to moderate levels of serum anti-TPO antibodies can be found in some other autoimmune pathology (eg systemic lupus erythematosus or Sjogren syndrome) and, rarely, in apparently healthy subjects (especially elderly women). Anti-TPO antibodies are more sensitive in diagnosis of thyroid autoimmune diseases than anti-thyroglobulin (anti-TG) antibodies. However, in some cases anti-TG positive sera may be negative for anti-TPO. Therefore, combined determination of both types of anti-thyroid antibodies (anti-TPO + anti-TG) provides a more sensitive laboratory diagnostic tool for thyroid autoimmunity.

### PRINCIPLE OF THE ASSAY

The present test is a sandwich enzyme immunoassay on solid phase (ELISA). Patient specimen is placed into the microwells of a microplate coated with antigen (TPO). Antibodies, eventually present in the sample, are captured by the antigen onto the microwell surface, while unbound material is removed by washing. During a second incubation, anti human IgG antibodies labelled with peroxidase enzyme (HRP), are added into the microwells. After incubation unbound labelled antibodies are removed by washing, while the remaining enzymatic activity bound to the microwell surface is detected by addition of chromogen-substrate solution. Developed colour, revealed at 450 nm, is directly related to the quantity of anti-TPO antibodies present in the specimen. The optical density values measured for the standards are used to generate a calibration curve from which the analyte concentration in the sample can be ascertained.

### KIT CONTENT

#### 1. Reagent A – Microplate

12x8 strips.

8 wells breakable strips, coated with Thyroid peroxidase. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature **before** use, to prevent any moisture formation inside the bag.

#### 2. Reagent A1 – Preliminary plate

12x8 strips.

Polystyrene plate for the first samples dilution with Sample Diluent 1.

#### 3. Reagent B – Enzymatic Tracer

1 vial of 12 ml.

Ready to use liquid reagent containing human IgG antibodies labelled with Horseradish peroxidase (HRP) diluted in a protein-stabilized matrix containing gentamycin sulphate 0,004% and Proclin 300 0,05% as preservatives.

#### 4. Reagent C – Washing Solution 25x

1 vial of 50 ml.

Concentrated solution to be diluted 1:25 with distilled water.

#### 5. Reagent D/E – Chromogen/Substrate

1 vial of 12 ml.

Ready to use solution containing Tetramethylbenzidine (TMB) in Citric buffer and H<sub>2</sub>O<sub>2</sub>.

**Avoid light exposure.**

#### 6. Reagent F – Stop Solution

1 vial of 15 ml.

Ready to use solution containing a solution of Sulphuric acid 0.2 M

**Avoid any skin contact.**

#### 7. Anti-TPO Standards

5 vials of 1.0 ml each.

Ready to use liquids containing anti-TPO at the following concentrations (approximate):

**S<sub>0</sub>**: 0 IU/ml, **S<sub>1</sub>**: 25 IU/ml, **S<sub>2</sub>**: 100 IU/ml,

**S<sub>3</sub>**: 250 IU/ml, **S<sub>4</sub>**: 500 IU/ml.

Exact concentrations are stated on the label, they are lot-specific.

Human sera used for the standards are diluted in a buffer solution containing Sodium Azide 0.1% and Phenol 0.1% as preservatives.

#### 8. Anti-TPO Control

1 vial of 1.0 ml.

Ready to use liquid reagent containing diluted human serum at a defined content of anti-TPO with Sodium Azide 0.1% and Phenol 0.1% as preservatives.

Concentration range is stated on the label.

#### 9. Reagent G1 – Sample diluent 1 (preliminary)

1 vial containing 12 ml.

Blue-violet ready to use liquid containing Sodium Azide 0.1% and Phenol 0.1% as preservatives.

#### 10. Reagent G2 – Sample diluent 2

1 vial containing 12 ml.

Pink ready to use liquid containing Sodium Azide 0.1% and Phenol 0.1% as preservatives.

#### 11. Cardboard sealers

2 cardboard sealers to be used to cover the plate during the incubations.

#### 12. Package insert: instruction for use GD7320 00 it/ing.

## MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121°C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5 % for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

## STORAGE AND STABILITY OF THE KIT

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before restore at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

## AUXILIARY MATERIALS

- Semi automatic pipettes of 25-1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filter of 450 nm (620- 630 nm).
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

## SAMPLES

Serum or plasma (in ACD heparin). Specimens can be stored up to 48 hours at 2-8 °C before testing; for a long storage, the specimens should be frozen at -20 °C. Repeated freezing/thawing should be avoided. Turbid, hemolytic, lipemic, or contaminated microbiologically samples should be avoided.

## REAGENTS PREPARATION

- WASHING SOLUTION: dilute 1:25 with distilled or ELISA grade water (e.g: 1 ml of Reagent C + 24 ml of distilled water) and mix carefully before use. The diluted washing solution can be stored for 14 days at room temperature or 28 days at +2-8 °C. It is recommended to store diluted washing solution at room temperature for immediate use.

## WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 3-5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

## ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards, control and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards, control and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blinking" of the instrument is to be carried out in the Standard 0 U/ml.

### ASSAY SCHEME

- Put the desired number of microstrips into the frame.
- Dilute samples 1:10 in the preliminary plate A1 with Reagent G1** (Blue-violet Buffer), (ex.: 10 µl of sample + 90 µl of Reagent G1). Do not dilute Standards and Control. After dilution colour should change to blue-green.  
Follow the scheme:

|                       | Microwells coated with human TG   |                  |        |
|-----------------------|---|------------------|--------|
|                       | REAGENTS  | Standard/Control | Sample |
| First incubation      | Standard/Control  | 100 µl           | -      |
|                       | Sample Diluent 2 (G2 - Pink buffer)   |                  | 90 µl  |
|                       | Prediluted Sample   | -                | 10 µl  |
|                       | - Cover the strips with cardboard sealer<br>- Incubate <b>60 minutes at 18-25 °C</b>  |                  |        |
| Wash                  | - Peel out the cardboard sealer and aspirate the reaction solution from all wells<br>- Rinse 3 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid |                  |        |
| Second incubation     | Reagent B (Tracer)  | 100 µl           | 100 µl |
|                       | - Cover the strips with cardboard sealer<br>- Incubate <b>60 minutes at 18-25 °C</b>  |                  |        |
| Wash                  | - Peel out the cardboard sealer and aspirate the reaction solution from all wells<br>- Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid |                  |        |
| Colorimetric reaction | Reagent D/E (Chromogen-Substrate)   | 100 µl           | 100 µl |
|                       | - Cover the strips with cardboard sealer<br>- Incubate <b>10-15 minutes at 18-25 °C, avoiding light exposure</b>  |                  |        |
|                       | Reagent F (Stop Solution)   | 100 µl           | 100 µl |
|                       | Read the absorbance of each well against Blank (Standard 0) at 450 (and 620-630 nm)   |                  |        |

### CALCULATION OF RESULTS

- Calculate the mean value of the OD 450 nm obtained for each duplicate.
- Subtract blank value (Standard 0) to the mean OD 450 nm values of standards, control(s) and sample.
- Draw a standard curve by plotting the absorbances of the standards with the corresponding concentrations. A point-by-point method for data reduction is recommended. Alternatively, the calculation system of the microplate reader software can be used.
- Calculate the concentrations of control(s) and samples from the obtained standard curve.

### QUALITY CONTROL

Anti-TPO Control concentration should fit into the established range stated on the labels.

It is important to always include, within the test procedure, commercial controls with known anti-TPO concentrations for validating the accuracy and the precision of the test. The test results are valid only if all controls are within the specified ranges.

### VALIDITY OF THE TEST

For the test to be valid the following criteria must be met:

- Standard 4 (500 IU/ml): OD 450 nm:  $\geq 1.3$
- Calculated value of Control should be within the established range stated on the label.

### Example of calculation

Example of calibration curve, do not utilize for the calculation of results.

| Standard (IU/ml) | OD 450 nm - blank |
|------------------|-------------------|
| S0 (0 IU/ml)     | 0.050             |
| S1 (25 IU/ml)    | 0.339             |
| S2 (100 IU/ml)   | 1.259             |
| S3 (250 IU/ml)   | 2.065             |
| S4 (500 IU/ml)   | 2.510             |

### EXPECTED VALUES

Based on data obtained by AMS, the following normal ranges are recommended (see below).

However, it is recommended that each laboratory establish its own reference range.

| Sex, age           | IU/ml       |             |
|--------------------|-------------|-------------|
|                    | Lower limit | Upper limit |
| Males              | -           | 30          |
| Females            | -           | 30          |
| Females > 50 years | -           | 50          |

**Note:** Therapeutic consequences should not be based the results obtained by this method alone; all available clinical and laboratory findings should be used by physicians to elaborate therapeutically measures.

## ANALYTICAL PERFORMANCES

### Sensitivity

The lowest detectable concentration of anti-TPO is 2 IU/ml.

### Specificity

Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant.

### Precision

#### a. Intra-Assay Precision

Three samples were assayed 15 times each on the same calibration curve. The results (in IU/ml) are tabulated below:

| Sample | Mean (IU/ml) | SD   | CV% |
|--------|--------------|------|-----|
| 1      | 365.0        | 25.2 | 6.9 |
| 2      | 213.8        | 15.3 | 7.2 |
| 3      | 123.0        | 8.5  | 6.9 |

#### b. Inter-Assay Precision

Two samples were assayed 2 times 3 runs a day. The results (in IU/ml) are tabulated below:

| Sample | Mean (IU/ml) | SD   | CV% |
|--------|--------------|------|-----|
| 1      | 345.7        | 19.9 | 5.8 |
| 2      | 201.3        | 15.2 | 7.5 |
| 3      | 119.1        | 8.7  | 7.3 |

### Accuracy

The present kit was compared with a reference Chemiluminiscent microparticle immunoassay. The total number of analyzed samples was 135.

The following linear regression curve was calculated:

$$y = 0.92x + 10.2 \quad r = 0.95$$

## PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentrations of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

### Waste Management

Please refer to local legal requirements.

## REFERENCES

1. Volpe R., "Autoimmune disease of the endocrine system", Boca Raton FL, CRC Press (1990).
2. Volpe R., Clin Chem, Vol. 40, 2132 (1994).
3. Degroot LJ, "Heterogeneity of human antibodies to TPO Thyroperoxidase", Thyroid Autoimmunity, 207, 177-182 (1990).