Anti-TG

Enzyme immunoassay for the quantitative determination of Anti-TG in human serum or plasma

Only for in-vitro diagnostic use

Product Number: DNOV115 (96 Determinations)
1. INTRODUCTION

Thyroglobulin (TG) is a protein produced by and used entirely within the thyroid gland. Thyroglobulin is used by the thyroid gland to produce the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Thyroglobulin synthesis is stimulated at the transcriptional level by thyroid-stimulating hormone (TSH).

Thyroglobulin (TG) is a well-known target for autoantibodies occurring in thyroid autoimmunity (Graves' disease and Hashimoto's thyroiditis). Anti-TG antibodies mostly belong to the IgG class. Low to moderate levels of anti-TG antibodies can be found in sera of other autoimmune patients (e.g., systemic lupus erythematosus or Sjogren syndrome). In some cases, anti-TG positive sera may show negativity for other type of anti-thyroid antibodies - anti-TPO. Therefore, combined determination of both types of anti-thyroid antibodies (anti-TPO + anti-TG) provides most sensitive laboratory diagnostic tool for thyroid autoimmunity. Separately from autoimmunity, anti-TG antibodies may develop in patients suffering from thyroid cancer. High level of anti-TG in such patients may interfere with correct determination of serum thyroglobulin, which serves as tumour marker for therapy control in this group of patients.

2. INTENDED USE

Immunoenzymatic colorimetric method (ELISA) for quantitative determination of Anti-TG in serum or plasma.

3. PRINCIPLE OF THE ASSAY

This test is based on two-site sandwich enzyme immunoassay principle. The microplates are coated with Thyroglobulin. In the first step, antibodies from the sample bind to the coated antigen on the microwell surface. Unbound material is removed by a washing procedure. Second antibodies directed against human IgG and labelled with horseradish peroxidase, are then added to the microwells. A second washing step is performed. Then the immune complex is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of this product is proportional to the amount of Anti-TG in samples and standards. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied
- Coated Microplate: 12 breakapart 8-well snap-off strips coated with Thyroglobulin; in resealable aluminium foil.
- Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0,15 mol/l (avoid any skin contact).
- Sample Diluent: 1 bottle containing 100 ml phosphate buffer (50 mM, pH 7.4, 1 g/L BSA).
- Conjugate: 1 bottle containing 15 ml of horseradish peroxidase labelled anti-human IgG.
- TMB Substrate Solution: 1 bottle containing 15 ml 3, 3', 5, 5'-tetramethylbenzidine (H₂O₂-TMB 0.25 g/l) (avoid any skin contact).
- Wash Solution conc.: 1 bottle containing 20 ml 50x concentrated wash solution (NaCl 9 g/l and Tween-20 1 g/l).
- Negative Control: 1 bottle containing 1.2 ml control solution, ready to use, < 4 U/ml.
- Positive Control: 1 bottle containing 1.2 ml control solution, ready to use, > 4 U/ml.
- Anti-TG Standards: 6 bottles, 1.2 ml each. The standards have the following concentrations in arbitrary units (U):
  - Standard 0: 0 U/ml
  - Standard 1: 2 U/ml
  - Standard 2: 4 U/ml
  - Standard 3: 8 U/ml
  - Standard 4: 32 U/ml
  - Standard 5: 128 U/ml

4.2. Materials supplied
- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- automatic dispenser

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2…8 °C in the original container.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!
6.1. Coated Microplate

The ready to use break apart snap-off strips are coated with Thyroglobulin. Store at 2…8 °C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.

6.2. Conjugate

The bottle contains 15 ml of a ready to use solution with anti-human IgG conjugated with HRP.

6.3. Standards

The standards are ready to use.

6.4. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2…8°C in the dark. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6.5. Stop Solution

The bottle contains a ready to use solution of 1 M sulphuric acid solution (Xi, R 36/38).

6.6. Wash Solution

Dilute the concentrated Wash Solution with distilled water to 1000 ml and store at 2…8°C for at least 30 days or until the expiry date printed on the label.

6.7. Sample Diluent

The sample diluent is ready to use.

7. SPECIMEN COLLECTION AND PREPARATION

For determination of Anti-TG serum or plasma are the preferred sample matrixes. The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum or plasma from the cells by centrifugation after clot formation. Samples may be stored refrigerated at 2…8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted. Do not use microbiologically contaminated samples, as highly lypemic or hemolized samples.

7.1. Sample dilution

Samples have to be diluted prior to use. Add 10 µl serum/plasma to 1000 µl diluted sample diluent.

8. ASSAY PROCEDURE

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

<table>
<thead>
<tr>
<th>Well</th>
<th>Standard/Control</th>
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<tbody>
<tr>
<td>1</td>
<td>for blank</td>
</tr>
<tr>
<td>2</td>
<td>for standard 0</td>
</tr>
<tr>
<td>2</td>
<td>for standard 1</td>
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<tr>
<td>2</td>
<td>for standard 5</td>
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<tr>
<td>2</td>
<td>for negative control</td>
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<tr>
<td>2</td>
<td>for positive control</td>
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</tbody>
</table>

It is recommended to determine standards and patient samples in duplicate. Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 100 µl standards and diluted samples and controls into their respective wells. Leave A1 for blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 30 min at room temperature (22…28 °C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted Wash Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5Sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

*Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.*
5. Dispense 100 µl conjugate into all wells except blank and cover them with the foil supplied in the kit.

6. **Incubate for 30 min at room temperature (22…28 °C).**

7. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted Wash Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

8. Dispense 100 µl TMB Substrate Solution into all wells.

9. **Incubate for exactly 15 min at room temperature (22…28 °C) in the dark.**

10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.

11. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

### 9. RESULTS

#### 9.1. Standard Curve

For Anti-TG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable.

#### 9.2. Recommended Lin-Log Plot

First calculate the averaged optical densities for each standard well. Use lin-log graph paper and plot the averaged optical density of each standard versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The standard points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the standard curve by interpolation.

#### 9.3. Reference values

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-TG tests:

- **normal:** < 4 U/ml
- **elevated:** ≥ 4 U/ml

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.

It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-TG.

### 10. SPECIFIC PERFORMANCE CHARACTERISTICS

#### 10.1. Sensitivity

Comparison test against a commercial reference kit, assayed on 66 serums (26 of them positive sera and 40 negative sera) shows a 88.5% sensitivity.

#### 10.2. Specificity

Comparison test against a commercial reference kit, assayed on 66 serums (26 of them positive sera and 40 negative sera) shows a 95% specificity.

#### 10.3. Detection limit

The lowest concentration of anti-TG that can be distinguished from standard 0 is about 0.31 U/ml with a confidence limit of 98%.

### 11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

### 12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg by FDA cleared methods and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

**WARNING:** Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

### 12.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

### 13. LITERATURE


### 14. ORDERING INFORMATION

| Prod. No.: | DNOV115 | Anti-TG Determination (96 Determinations) |
SCHEME OF THE ASSAY

Anti-TG

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

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<tr>
<th></th>
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<th>Standard 0 - 5</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Sample</th>
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<tr>
<td>Standard 0-5</td>
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<tr>
<td>Negative Control</td>
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<td>100 µl</td>
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<tr>
<td>Positive Control</td>
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<td>-</td>
<td>100 µl</td>
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<tr>
<td>Sample</td>
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<td>100 µl</td>
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</tbody>
</table>

Cover wells with foil supplied in the kit.

**Incubate for 30 min at 22...28 °C.**
Wash each well three times with 300 µl diluted Wash Solution.

<table>
<thead>
<tr>
<th></th>
<th>Conjugate</th>
<th>TMB Subst.</th>
<th>Stop Solution</th>
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<tbody>
<tr>
<td></td>
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Cover wells with foil supplied in the kit.

**Incubate for 30 min at 22...28 °C.**
Wash each well three times with 300 µl diluted Wash Solution.

**Incubate for exactly 15 min at room temperature.**

Photometric measurement at 450 nm

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