

Test Instructions

Enzyme Immunoassay for the Determination of Anti-Thyroperoxidase-Antibodies

Catalogue-No.: TC 85020

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

Reagents from different test kit lots must not be mixed.

1. Clinical Use

Autoantibodies against thyroid gland antigens play an important role in the pathogenesis, diagnostics and differential diagnostics of diseases of the thyroid gland caused by autoimmune reactions. These autoantibodies can frequently be detected in Morbus Basedow as well as in atrophic and hypertrophic conditions of chronic lymphocytic thyroiditis.

This means they are an important criterion for the differentiation of autoimmune and non autoimmune diseases of the thyroid gland. But it should be taken into consideration that antibodies of the thyroid gland are found with a frequency of 2 - 11 % in healthy people and that the incidence among the female sex is even higher.

2. Principle of Test

The test is based on the covalent binding of thyroperoxidase (TPO) to chemical activated microtiter strips (patent pending) and subsequent binding of anti-TPO antibodies from patient serum. The bound antibodies are detected with an peroxidase-labelled secondary antibody which is directed against human IgG. After addition of a substrate solution, a color stain develops, the intensity of which is proportional to the concentration and/or the avidity of the antibodies.

3. Material Provided

- TPO coated microtiter strips (1 x 8), breakable	12 strips
- standards; ready to use,	1 vial each à 750 µl
1: 11.5 WHO-IU/ml	
2: 46 WHO-IU/ml	
3: 183 WHO-IU/ml	
4: 732 WHO-IU/ml	
5: 2930 WHO-IU/ml (contain sodium azide)	
- negative control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- positive control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- washing buffer concentrate (10x), (contains thimerosal)	1 bottle 50 ml
- dilution buffer concentrate (5x), (contains thimerosal)	1 bottle 22 ml
- peroxidase conjugate, anti-human IgG, concentrate (100x),	1 vial 200 µl
- peroxidase substrate solution, (TMB), ready to use,	1 bottle 12 ml
- stopping solution, H ₂ SO ₄ , ready to use,	1 bottle 12 ml

4. Preparation of Reagents

Allow the kit to reach room temperature!

4.1 Preparation of Washing Buffer

If any of the salts have been crystallized they should be resolved before use. Dilute 1 unit washing buffer concentrate with 9 units distilled water. The ready to use buffer is stable for 6 weeks stored at 2 - 8 °C and can also be used in the IMTEC-Anti-Thyroglobulin-EIA (Cat.-No. TC 85010).

4.2 Preparation of Dilution Buffer

Dilute 1 unit dilution buffer concentrate with 4 units distilled water. If any of the salts have been crystallized they should be resolved before use. The ready to use buffer is stable for 6 weeks stored at 2 - 8 °C.

4.3 Preparation of Standards and Control Sera

The standards and the control sera are ready to use.

4.4 Preparation of Sera

Use serum samples freshly collected or freeze the samples at minus 20 °C. Allow sera to reach room temperature (30 min). Dilute sera 1:100 with dilution buffer (10 µl serum with 1 ml buffer). The diluted serum samples can also be used in the IMTEC-Anti-Thyroglobulin-EIA (Catalogue-No. TC 85010).

4.5 Preparation of Conjugate

The daily required amount of conjugate solution should be prepared freshly. Do not use polystyrene tubes to prepare the conjugate dilution.

Dilute conjugate 1:100 with ready to use dilution buffer (for one plate: 100 µl conjugate with 10 ml buffer, for two strips: 20 µl conjugate with 2 ml buffer). Remaining solution should be disposed.

4.6 Preparation of Substrate

The TMB-substrate solution is ready to use. Used substrate bottle should be closed carefully. Store substrate solution at 4 - 8 °C protected from light.

4.7 Microtiter Strips

The strips are ready to use. Unused strips should be stored in the lockable original bag at 2 - 8 °C.

4.8 Stopping Solution

H₂SO₄ (caution!)

5. Test Procedure

1. **Pipette 100 µl serum dilution** or (undiluted) standards and control sera into each well, for blanks use ready to use dilution buffer instead of serum dilution, seal wells with adhesive foil
2. **Incubate for 1 hour** at room temperature (RT)
3. **Rinse off the wells 3 x** with min. 200 µl washing buffer per well
4. **Pipette 100 µl of conjugate dilution** into each well, seal wells with adhesive foil

5. **Incubate for 30 minutes** at RT

6. **Rinse off the wells 3 x** with min. 200 µl washing buffer per well

7. **Pipette 100 µl substrate solution** into each well

8. **Incubate for 10 min** at RT in the dark. At a room temperature higher than 25 °C the substrate incubation time should be shortened. The minimum substrate incubation time must be 5 minutes.

9. **Pipette 100 µl stopping reagent** into each well

10. **Measure at 450 nm** within the next 30 min after stopping

6. Interpretation of Results

Calibrate measured absorbance against concentrations/units of standards (11,5 WHO-IU/ml, 46 WHO-IU/ml, 183 WHO-IU/ml, 732 WHO-IU/ml, 2930 WHO-IU/ml), (semi-log). Read the units of the examined sera from the standard curve directly.

Results lower than 80 WHO-IU/ml are considered negative. Results from 80 to 150 WHO-IU/ml indicates a slightly positive reaction of the patient serum or are just in the range.

Results above 150 WHO-IU/ml (cut off value) are considered positive.

To prove the test function the value of the positive control serum has to be within the range (see label on the vial). The negative control result has to be lower than 80 WHO-IU/ml.

Precautions

For in vitro diagnostic use only.

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



IMTEC Immundiagnostika GmbH

Robert-Rössle-Straße 10

D-13125 Berlin

GERMANY

Tel.: +49(0)30 94 89 36 00

Fax: +49(0)30 94 89 36 15