abia TPO Ab

REF DK.014.01.3

IVD

CE

Note: Changes highlighted ★

abia

Intended use

abia TPO Ab enzyme immunoassay for the quantitative determination of antibodies to thyroid peroxidase (anti-TPO) concentration in human serum.

The assay is intended for aid in the assessment of thyroid status and diagnosis of thyroid disease. For professional use only.

Clinical value

Antibodies to thyroid peroxidase (anti-TPO) have been shown to be characteristically present from patients with Hashimoto thyroiditis (95%), iodopathic myedema (90%) and Graves Disease (80%).

In fact 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction. This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction.

Principle of the test

abia TPO Ab is an two-step indirect assay based on microwells coated with human thyroid peroxidase (TPO). The conjugate is a mixture of HRP-labeled monoclonal anti-human-IgG antibodies.

Serum samples are added to the wells and if anti-TPO antibodies are present in a sample, they form stable complexes with human TPO immobilized on the wells.

Then the antigen-antibody complexes are identified by the addition of HRP labeled anti-human-IgG conjugate.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue color which is converted to yellow after the reaction has been stopped with sulphuric acid.

The color intensity is directly proportional to the concentration of the anti-TPO antibodies in the specimen and can be read at 450 nm.

Kit contents

	S	
TPO Ag coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with human thyroid peroxidase (TPO)
Conjugate	1 × 12 ml	ready to use; HRP-labeled anti-human IgG mAb; transparent or slightly opalescent yellow liquid
Sample diluent 1	1 × 12 ml	buffer for the 1st samples dilution in preliminary plate; transparent or slightly opalescent blue-violet liquid
Sample diluent 2	1 × 12 ml	buffer for the 2nd samples dilution in TPO Ag coated plate; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 1.0 ml	protein based calibrator not containing anti-TPO; colorless or pale yellow liquid
Calibrator 1	1 × 1.0 ml	human serum based calibrator containing anti-TPO in concentration approx. 25 IU/ml; colorless or pale yellow liquid
Calibrator 2	1 × 1.0 ml	human serum based calibrator containing anti-TPO in concentration approx. 100 IU/ml; colorless or pale yellow liquid
Calibrator 3	1 × 1.0 ml	human serum based calibrator containing anti-TPO in concentration approx. 250 IU/ml; colorless or pale yellow liquid
Calibrator 4	1 × 1.0 ml	human serum based calibrator containing anti-TPO in concentration approx. 500 IU/ml; colorless or pale yellow liquid
Control serum	1 × 1.0 ml	human serum based control containing anti-TPO; colorless or pale yellow liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colorless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H_2O_2; colorless liquid
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colorless liquid
Protective film	2	
Plastic dish	2	
Zip-lock plastic bag	1	

The calibrators were calibrated using a 1st IS 66/387. Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis.
All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.
Once opened, the components should be used within one month. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- preliminary plate for samples pre-dilution with the sample diluent 1 reagent
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- human origin material used in the preparation of the calibrators and control serum has been tested by CE-marked tests and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2)
- as no known test method can offer complete assurance that infections agents are absent, handle reagents and samples as if capable of transmitting infections disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- pooled specimens must not be used since the accuracy of test with such specimens has not been validated
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section "Washing procedure"
- for the description of test procedure with the automated analyzers see section "Automated analyzers"

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- repeat 3 times in the step 5 and 5 times in the step 8 of the test procedure
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia TPO Ab for the quantitative determination of antibodies to thyroid peroxidase (anti-TPO) concentration in human serum

1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in zip-lock plastic bag. Do not remove desiccant.
2	Add 90 µl of sample diluent 1 to premilinary plate (not provided). Add 10 µl of samples to be tested into appropriate wells. Mix the contents of the wells by gentle pipetting. The color intensity of the solution should change. No change of the color can be observed if no serum added to the well.
3	Analyse each calibrator, control serum and samples in duplicate. Add 100 µl of calibrators 0 - 4, control serum into appropriate wells. Add 90 µl of sample diluent 2 in rest of the wells. Add 10 µl of prediluted samples to be tested from preliminary plate in the wells with sample diluent 2. Final dilution ratio is 1:100. Mix the contents of the wells by gentle pipetting, then cover the plate with protective film. The total time should not exceed 10 min.
4	Incubate for 60 minutes at room temperature 20–25 °C.
5	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 3 times. Use double aspiration in the final step where possible.
6	Add 100 µl conjugate into each well. Mix the contents of the wells for 30 seconds by careful tapping on the edge of the plate, then cover the plate with protective film.
7	Incubate for 60 minutes at room temperature 20–25 °C.
8	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
9	Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 10–15 minutes at 20–25 °C.
10	Add 150 µl of stopping reagent into each well. Mix gently for 5–10 sec.
11	Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section "Test procedure" and ensure all requirements described in section "Precautions" are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of control serum should be within established range.
The absorbance (OD) of calibrator 4 should be greater than 1.300.

Calculate procedure

1 Calculate the mean optical density of each calibrator duplicate.

2 Calculate the mean optical density of each samples duplicate.

3 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.

4 Read the values of the unknowns directly off the calibration curve.
If immunoassay software is being used a 4-parameter curve is recommended.

Example	OD 1	OD 2	Mean OD	Value, IU/ml
Calibrator 0	0.048	0.051	0.051	0.00
Calibrator 1	0.350	0.328	0.339	25.00
Calibrator 2	1.295	1.222	1.259	100.00
Calibrator 3	2.087	2.042	2.065	250.00
Calibrator 4	2.504	2.515	2.510	500.00
Sample	0.778	0.760	0.769	60.00

This data is for illustration only and should **not be used** to calculate of samples. Each user should obtain his or her own data and standard curve.

Performance characteristics

Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 12 replicate analyses additional 2 x SD. Limit of detection defined at 2.00 IU/ml.

Specificity

No cross-reactivity to ANA, DNA, thyroglobulin and rheumatoid antibodies was observed with this assay.

Precision	Mean, IU/ml	SD	CV, %
Intra-assay, sample 1	49.90	2.77	5.60
Inter-assay, sample 1	50.50	0.74	1.50

Accuracy

The assay was compared with a other immunoassay as a reference test. The total number of specimens was 156. The values ranged from 0.00 to 769.70 IU/ml. The concordance was 98.10%.

Expected normal value

A normal range of less than 30 IU/ml anti-TPO was obtained by testing serum samples from 250 individuals determined as normal by abia TSH and abia fT4 assays.

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of test

- assay was validated only for the determination of anti-TPO antibodies in human serum
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient
- the presence of autoantibodies to TPO is confirmed when the serum level exceeds 30 IU/ml. The clinical significance of the result, coupled with anti-thyroglobulin activity, should be used in evaluating the thyroid condition. However, clinical inferences should not be solely based on this test but rather as an adjunct to the clinical manifestations of the patient and other relevant tests
- about 10% of asymptomatic specimens may present with anti-TPO autoantibodies reflecting the prevalence in apparently healthy populations. The prevalence of anti-TPO may also depend on age, gender and geographic region of the selected population

References

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

Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalogue number
LOT	Batch code
YYYY-MM-DD	Expiry date
2°C 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
Σ n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

Hazard and precautionary statements for certain kit components

Warning

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Warning

Conjugate

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

2018-03-27 IU.014.01.3/0 DRAFT

AB Diagnostic Systems GmbH
Sportfliegerstraße 4
12487 Berlin
Germany

+49 30 208 987 160
+49 30 208 987 199
info@ab-ds.de
www.ab-ds.de