AB Diagnostic Systems

REF **DK.014.01.9** Σ **96**

CE

IVD **For *In vitro* Diagnostic Use**

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

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 assays (breakable wells) including controls; partial use of the kit is possible; can be used for manual protocol.

I. INTENDED USE

The abia TPO Ab kit is intended for the quantitative determination of anti-Thyroid Peroxidase concentration in human serum by a microplate enzyme immunoassay.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

Antibodies to thyroid peroxidase 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. Antibodies to thyroid peroxidase (TPO) are mainly IgG. Anti-TPO may be present in healthy individuals in a concentration up to 30 IU/ml. The anti-TPO levels above this concentration are indicative of an autoimmune disorder. The determination of anti-TPO level can be used for the diagnosis of autoimmune thyroid diseases (Graves' disease, Hashimoto's thyroiditis).

III. PRINCIPLE OF THE TEST

The abia TPO Ab plates are coated with human Thyroid Peroxidase. Serum to be tested is diluted and incubated with the precoated plate. In this step TPO specific antibodies are bound to the immobilized human Thyroid Peroxidase. Non specific antibodies are removed by washing. Anti-human IgG conjugated to horseradish peroxidase (HRP) is added and incubated. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex. Unbound conjugate is removed by washing. The presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a color change in TMB-Substrate solution.

IV. CONTENT OF THE KIT abia TPO Ab

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
TPO coated microtiter wells	Polystyrene stripped 96-well plate (breakable wells) coated with human Thyroid Peroxidase. Store at 2-8 °C until expiration date.	1 plate
Conjugate	Antibodies against human IgG, conjugated with HRP enzyme in a protein-stabilized matrix. Transparent or opalescent yellow color liquid. Preserving agent: 0.1% ProClin 300, 0.004% gentamicin sulfate, 0.10% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml

Calibrator 0 Calibrator 1 Calibrator 2 Calibrator 3 Calibrator 4	Five vials of anti-TPO Calibrators. Calibrators, human serum based, were calibrated using I International Standard 66/387. The anti-TPO concentration levels in Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis. Transparent or opalescent liquids, colorless or pale yellow. Preserving agent: 0.099% sodium azide, 0.10% phenol. Store at 2-8 °C until expiration date in tightly sealed vials.	5 vials 1.0 ml
Control Serum	Control serum with a difined quantity of anti-TPO. Exact concentration level is given on the label of the vial and in the Certificate of Analysis on a lot-specific basis. Transparent or opalescent liquid, colorless, or pale yellow. Preserving agent: 0.099% sodium azide, 0.10% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 1.0 ml
Sample Diluent 1	Buffer that is used for the first samples dilution in plate for preliminary dilution of sera before analysis. Transparent or slightly opalescent blue liquid. Preserving agent: 0.097% sodium azide, 0.10% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml
Sample Diluent 2	Buffer that is used for the second samples dilution in working plate for analysis. Transparent or slightly opalescent pink liquid. Preserving agent: 0.099% sodium azide, 0.10% phenol. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 12.0 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7). Transparent or slightly opalescent liquid, colorless or pale yellow, sediment may form that dissolves completely at 35-39 °C and shaking. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric acid buffer, containing H_2O_2 (0.01%). Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulfuric acid solution. Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 25.0 ml
Plate for preliminary dilution of sera	Polystyrene plate with transparent wells.	1 plate

Additionally the following may be included in the delivery set:

- a lid for polystyrene 96-well plates or a protective film for EIA plates;
- disposable tips;
- a plastic dish for liquid reagents;
- polyethylene bag with a Zip-Lock.

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. In case of label loss or labels/vials damage, vials should be disposed and **kit cannot be used.**
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various Conjugate or TMB-Substrate.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "in vitro diagnostic use".
- Human origin material used in the preparation of Control Serum and Calibrators have been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2), antigen p24 HIV-1.
- Certain reagents contain biological material of animal origin.

- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patients samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.
- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate contains ProClin 300.

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

Danger!

Stopping Reagent contains 0.2M sulfuric acid.

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm filter.
- Open type automated analyzer with 450 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Blood samples should be collected according to the current practices. Serum only may be used. Separate serum as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Store/transport the samples in accordance with the current regulatory documentation. If samples are to be stored/transported for a longer period of time, they must be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed bacterial growing, hemolysis, hyperlipidemia must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **TPO coated microtiter wells.** Each 12-strips plate (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of strips/wells required for the assay. Place the unused strips/wells back into the foil-lined bag; reseal the foil-lined bag in a Zip-Lock plastic bag. Do not remove desiccant.
- **Conjugate;**
- **Calibrators 0-4;**
- **Control Serum;**
- **Sample Diluent 1;**
- **Sample Diluent 2;**
- **TMB-Substrate;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial.

The required volumes of Working Washing Solution for the certain number of strips or plate are tabulated in Table 2.

Table 2

Number of strips to be used		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1 well**
Working Washing Solution	Washing Solution (×25), 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	0.2
	Distilled or deionized 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	4.8

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature for 30 min.

1. Pipette 90 µl of Sample Diluent 1 to plate for preliminary dilution of sera and add 10 µl of the samples (first samples dilution ratio is 1:10). Carefully mix fluid in wells by gentle pipetting. The color intensity of the solution should change. If no change of the color is observed, then test result may be false, or there is no serum added to the well.

2. Pipette 100 µl of Calibrators and Control Serum in duplicates. Leave two wells for OD control of TMB-Substrate (blank).

3. Pipette 90 µl of Sample Diluent 2 to the rest of working plate wells and pipette 10 µl of the prediluted samples from plate for preliminary dilution of sera (final samples dilution ratio is 1:100). Carefully mix fluid in wells by gentle pipetting. Pipetting of samples should not extend beyond ten (10) minutes.

4. Cover the strips with a plate lid or a protective film and incubate for 60 minutes at room temperature (here 20-25 °C).

5. Aspirate the contents of the wells into the container with disinfecting solution. Wash the plate 3 times with the working Washing Solution. For this, pipette the working Washing Solution up to the top of the wells (not less than 300 µl per well). Then aspirate the liquid to a disinfectant container. If necessary, knock the plate out onto filter paper folded several times to remove the residual moisture.

It is recommended to use automated microtiter washer. Inadequate washing may adversely affect the accuracy of the assay.

6. Add 100 µl of Conjugate to all wells, except for the wells for OD control of TMB-Substrate.

7. Cover the strips with a plate lid or a protective film and incubate for 60 minutes at room temperature (here 20-25 °C).

8. Wash the wells 5 times as in step 5.

9. Pipette 100 µl of TMB-Substrate into each well.

10. Incubate for 10-15 minutes at room temperature in the dark.

11. Pipette 150 µl of Stopping Reagent into each well. Gently mix for 5-10 seconds.

12. Read the plate on microplate reader at 450 nm. Reading must be completed within 20 minutes after addition of the Stopping Reagent.

Scheme of the assay is represented in Annex.

13. Automated analyzers

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XV). For the instrumentation without established validated protocol follow the section “TEST PROCEDURE” and ensure all requirements described in the section “PRECAUTIONS” are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider "dead" volume of vials and containers used for loading working solutions in the EIA analyzer.

XI. CALCULATION OF RESULTS

1. Calculate the mean absorbance value of each calibrator duplicate.
2. 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.
3. Calculate the mean absorbance values for each specimen.
4. Read the values of the unknowns directly off the calibration curve, if immunoassay software is being used, a 4-parameter curve is recommended.
5. If a sample reads more than value of Calibrator 4 then dilute it with Sample Diluent 2. The result obtained should be multiplied by the dilution factor.

Typical Tabulated Data

Calibrator	OD 1	OD 2	Mean OD	Value (IU/ml)
0	0.048	0.051	0.050	0
1	0.350	0.328	0.339	25
2	1.295	1.222	1.259	100
3	2.087	2.042	2.065	250
4	2.504	2.515	2.510	500
Unknown	0.778	0.760	0.769	60

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

Test Validation

In order for the assay results to be considered valid the following criteria should be met:

1. **Blank OD**: The absorbance value should ≤ 0.1.
2. The absorbance (OD) of **Calibrator 4** should be ≥ 1.3.
3. Calculated values of **Control Serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia TPO Ab

1. Assay Dynamic Range

The range of the assay is between 0-500 IU/ml.

2. Analytical sensitivity

The lower detection limit is 2.0 IU/ml. The sensitivity was calculated by determining the variability of the 0 IU/ml serum calibrator and using the 2 SD (95% certainty) statistics.

3. Specificity

Interferences from ANA, DNA, thyroglobulin and rheumatoid antibodies were found to be insignificant.

4. Intra-Assay Precision

One sample was assayed 9 times each on the same calibrator curve. The results (in IU/ml) are tabulated below:

Sample	Mean	SD	CV, %
1	49.9	2.7697	5.6

5. Inter-Assay Precision

One sample was assayed 4 times on the different calibrator curves. The results (in IU/ml) are tabulated below:

Sample	Mean	SD	CV, %
1	50.5	0.7411	1.5

6. Expected normal value

A normal range of less 30 IU/ml anti-TPO was obtained by testing serum specimens from 350 individuals determined as normal by abia fT4 and abia TSH assays. The measured anti-TPO levels did not exceed 30 IU/ml in 99.2% of cases. It is strongly recommended that each laboratory should determine its own normal range values.

7. Concordance

The kit abia TPO Ab was compared to another commercialy available immunoassay as a reference test. The total number of specimens was 156. Concordance = 98.1% (153 from 156).

XIII. LIMITS OF THE TEST

1. Highly lipemic, hemolyzed or grossly contaminated specimens should not be used.
2. It is important that the time of reaction in each well is held constant for reproducible results.
3. Any improper handing of samples or modification of this test might influence the results.
4. If more than 1 plate is used, it is recommended to repeat the dose response curve.
5. Do not touch the bottom of the wells.
6. 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.
7. 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.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

- **Expiry date is indicated on the packaging**. Storage and transportation conditions for the kit, conditions and terms of storage for working solutions and unused reagents are specified in Table 3.
- Transportation should be done at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 3

1	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited.		
2	**Transportation conditions**		
	at 2-8 °C		
	at 9-20 °C	not more than during ten (10) days	
3	Conditions and terms of storage for working solutions Keep in a dark dry place and in a chemically neutral vial.		
	Working Washing Solution	at 2-8 °C	For up to 28 days
		at 18-24 °C	For up to 14 days
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C.		
	TPO coated microtiter wells	Place the unused strips/wells back into the bag, reseal the foil-lined package in Zip-Lock plastic bag. Do not remove desiccant.	Until the kit expiration date
	Washing Solution, Stopping Reagent, Sample Diluent 1, Sample Diluent 2	Close the vials tightly with screw caps and stored them in the manufacturer's package.	Until the kit expiration date
	Calibrators 0-4, Control Serum, Conjugate, TMB-Substrate	Close the vials tightly with screw caps and stored them in the manufacturer's package.	For two months

XV. GUARANTEE

- Manufacturer guarantees conformity of the product to the requirements of regulatory and technical documentation.
- Quality and safety of the kit is guaranteed within established shelf life.
- Please contact Manufacturer if you have any questions.

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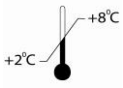
XVI. REFERENCES

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).

XVII. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)		Storage temperature limitation
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM	IVD	For in vitro diagnostic use
	Expiry date CCYY-MM-DD		Sufficient for
LOT	Batch code		Symbol “exclamation mark”
REF	Catalog number	**Warning!**	Signal word
	Fragile, handle with care		Symbol “corrosion”
	Keep away from sunlight	**Danger!**	Signal word
	Keep dry		Top

Annex

Scheme of the assay

1	**Add**	90 µl of Sample Diluent 1 and 10 µl of the samples (first samples dilution ratio is 1:10) conduct on the plate for preliminary dilution of sera
2	**Add**	100 µl of Calibrators and Control Serum in duplicates. Leave two wells for OD control of TMB-Substrate (blank). 90 µl of Sample Diluent 2 and 10 µl of the prediluted samples from plate for preliminary dilution of sera (final samples dilution ratio is 1:100) in duplicates to the rest of the wells
3	**Incubate**	60 min, at 20-25 °C
4	**Wash the plate**	Working Washing Solution, 300 µl, 3 times
5	**Add**	100 µl of Conjugate into all wells, to all wells except for the wells for OD control of TMB-Substrate (blank)
6	**Incubate**	60 min, at 20-25 °C
7	**Wash the plate**	Working Washing Solution, 300 µl, 5 times
8	**Add**	100 µl of TMB-Substrate into all wells
9	**Incubate**	10-15 min, at room temperature in a dark place
10	**Add**	150 µl of Stopping Reagent into all wells
11	**Mix**	5-10 seconds
12	**Read the optical density**	450 nm