AUTOZYME™ TAB

Anti-TPO antibodies

REF **Z2396**

Instructions for Use

IVD For in vitro diagnostics use only

Σ **96 Tests**

2°C 8°C **Store at 2 - 8°C**

EC REP

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CE

Kit Contents Symbols

CAL	Calibrators
CONTROL −	Negative Control
CONTROL +	Positive Control
BUF WASH	Wash Buffer
DIL SPE	Sample Diluent
CONJ	Conjugate solution
SUB	Substrate solution
STOP	Stop Solution
SORB	Solid Phase – Antigen Coated Wells

1. Intended Use

AUTOZYME™ TAB TPO is an enzyme immunoassay (EIA) for the screening and detection of auto-antibodies against thyroid peroxidase (TPO) in human serum. The assay is designed to be performed quantitatively and to be used as an aid in the diagnosis of thyroid disorder.

The standard values are traceable to the following reference preparation:
Anti-TPO - NIBSC 66/387

AUTOZYME™ TAB TPO has been specifically designed with automation in mind and can be adapted to automated immunoassay systems.

2. Background[1, 2]

Autoimmune thyroid gland disorders are characterised by the detection of anti-thyroid antibodies against TPO antigens. TPO has been identified as the specific antigenic determinant of the thyroid microsomal antigen[3].

The presence of auto-antibodies to thyroid antigens correlates with the degree of lymphocytic infiltration of the thyroid gland[4], the hallmark of Hashimoto's thyroiditis. Circulating auto-antibodies to TPO are present in the serum of over 90% of patients with thyroid autoimmune diseases such as Hashimoto's thyroiditis, Graves' disease, idiopathic hypothyroidism and sub-acute thyroiditis. In each case, the prevalence of anti-TPO antibodies is greater than that of anti-Tg antibodies.

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It is reported[5,6] that low levels of autoimmune antibodies predict at-risk pregnancy. Furthermore, a report[7] using an EIA test for anti-thyroglobulin and anti-recombinant TPO demonstrated a 100% increase in the rate of spontaneous miscarriage in women who had detectable serum thyroid auto-antibodies in their first trimester of pregnancy.

Thyroid auto-antibodies are detected using immunoassays such as passive haemagglutination, indirect fluorescence antibody (IFA), enzyme immunoassay (EIA) and radioimmunoassay (RIA) techniques. AUTOZYME™ TAB Anti-TPO uses recombinant human thyroid peroxidase which does not contain contaminating thyroglobulin and/or mitochondria found in other microsomal antigen preparations. This assay is in an EIA test format.

3. Principle

AUTOZYME™ TAB TPO employs a unique antigen-coated microwell technology, which is ideal for the batch-screening of large or small numbers of samples for anti -thyroid antibodies.

First Incubation
AUTOZYME™ TAB TPO wells are provided coated with purified antigen (recombinant TPO). When calibrators, controls or diluted sera are added, any anti -thyroid peroxidase antibodies present will bind to the well surface. The wells are then washed in buffer.

Second Incubation
Horseradish peroxidase-conjugated goat anti-human antibodies are added to the well, which will bind to any captured anti-thyroid antibodies. Unbound conjugate is removed by washing.

Third Incubation
A pale green substrate is then added to the wells. The intensity of the green colour formed is proportional to the concentration of anti-thyroid antibodies bound in the first incubation. The reaction is stopped with a low pH solution.

4. Kit Contents

6 vials calibrators (1.5 mL ready to use)

Calibrator	Anti-TPO (IU/mL)
1	0
2	25
3	100
4	250
5	1000
6	2500

1 vial wash buffer concentrate, 50 mL (x20)
1 vial sample diluent, 100 mL
1 vial conjugate (anti-human-IgG-HRP), 15 mL
1 vial substrate, 15 mL
1 vial stopping buffer, 15 mL
1 foil sachet containing 1 set of antigen-coated microwells
1 vial Negative Control (1.5 mL ready-to-use)
1 vial Positive Control (1.5 mL ready-to-use)
1 instruction leaflet
1 QC certificate

5. Storage

The kit should be stored at 2 - 8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight.

6. Sample Handling

AUTOZYME™ TAB TPO may be performed on human serum samples. Samples should be assayed within 24 hours of collection or stored frozen at -15°C or colder. Repeated freeze-thawing is not advisable. Do not heat treat samples.

7. Additional Reagents and Equipment

Deionised or freshly distilled water.
Precision micropipettes to deliver 10 - 1000 µL.
Multichannel micropipette or repeating dispenser to deliver 100 µL.
1000 mL measuring cylinder for reagent preparation.
Automated plate washer (optional).
96-well microplate reader with 405nm filter.
Software package (optional).

8. Procedural Precautions

Numbering of each strip is advised prior to commencing the assay.

Allow all reagents to equilibrate to room temperature (18 - 25°C) before use for a minimum of two hours.

Avoid the use of icteric, lipaemic or grossly haemolysed samples.

Always change tips between different calibrators, samples or control sera to prevent sample carry over.

Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate.

The substrate should be pale green. Any green colouration (absorbance >0.200) indicates substrate contamination and the substrate should be discarded. The well washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations).

Do not use the kit beyond the expiry date given on the label. Multiple re-use could increase the risk of reagent contamination.

9. Manual Assay Procedure

1. Prepare the wash buffer as follows: dilute contents of the **wash buffer concentrate** (x20) vial to 1000 mL with deionised water.
2. Dilute the patient samples 1/200 using the sample diluent e.g. 10 µL sample added to 1990 µL diluent. The **calibrators** and **kit controls** do not require dilution.
3. Remove the **antigen-coated microwells** from the foil sachet and seal any unrequired wells in the **foil sachet**, along with the desiccant sachet.
4. Dispense 50 µL of each **calibrator**, kit control or diluted patient sample into appropriate wells. Incubate for 30 minutes at room temperature (18 - 25°C). It is recommended that calibrators and controls be tested in duplicate and samples can be tested in singles or duplicate.
5. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Using the diluted wash buffer, wash the wells three times either with an automated plate washer set to at least 300 µL per well, or by adding 300 µL to each well and flicking out, gripping the frame on the long sides to retain the strips. Alternatively use a wash bottle. Blot the wells on absorbent material to remove any residual liquid.
6. Add 100 µL **conjugate** to each well and incubate for 30 minutes at room temperature (18 - 25°C).
7. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Wash the wells three times using the same procedure as in step 5.
8. Dispense 100 µL **substrate** into each well, ensuring that it is initially pale green and incubate for 30 minutes at room temperature.
9. Stop the reaction by adding 100 µL of **stopping buffer**.
10. Measure the absorbance at 405nm on a 96-well microplate reader.

10. Calculation of Results

For each assay, prepare a calibration curve by plotting mean absorbance against calibrator concentration on linear graph paper, and interpolate unknowns. Alternatively, use a computerised curve-fit program.

Any sample giving values above the calibrator range should be diluted and retested.

11. Quality Control

Quality control samples for anti-TPO antibodies are provided within the kits. Good laboratory practice requires that quality control samples be included in every run to check on assay performance.

Target ranges for the controls are quoted on the QC certificate. If either control value falls outside the quoted range, the results are invalid and the assay should be repeated.

12. Performance Data

a. Precision data:

		Anti-TPO Antibodies	
Intra-assay		IU/mL	CV%
Sample 1	(n=20)	36.1	3.6
Sample 2	(n=18)	1037.2	6.7
Sample 3	(n=20)	244.9	4.6
Inter-assay		IU/mL	CV%
Sample 1	(n=48)	250.7	5.4
Sample 2	(n=22)	104.1	3.9
Sample 3	(n=22)	1013.7	7.0

b. Minimum detectable concentration:

The minimum detectable concentration, defined as the concentration equal to 2 standard deviations from the mean of the sample diluent, was found to be less than:

anti-TPO antibodies 1 IU/mL

c. Reference values:

AUTOZYME™ TAB TPO was used to determine the anti-TPO levels of >200 serum samples from normal blood donors with no apparent abnormalities. The data was evaluated and the following ranges obtained:

Anti-TPO antibodies

Normal range	< 50 IU/mL
Borderline	50 - 75 IU/mL
Positive	> 75 IU/mL

It is advised that each laboratory establishes its own reference range.

13. Safety Precautions

For *in vitro* diagnostic use only.

For Professional Use only.

The **substrate** contains ABTS™ which is harmful if swallowed in copious amounts and may cause skin irritation if exposed for prolonged periods. In case of skin contact, wash with soap and water. Flush eyes with copious amounts of water.

The **calibrators and controls** contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence.

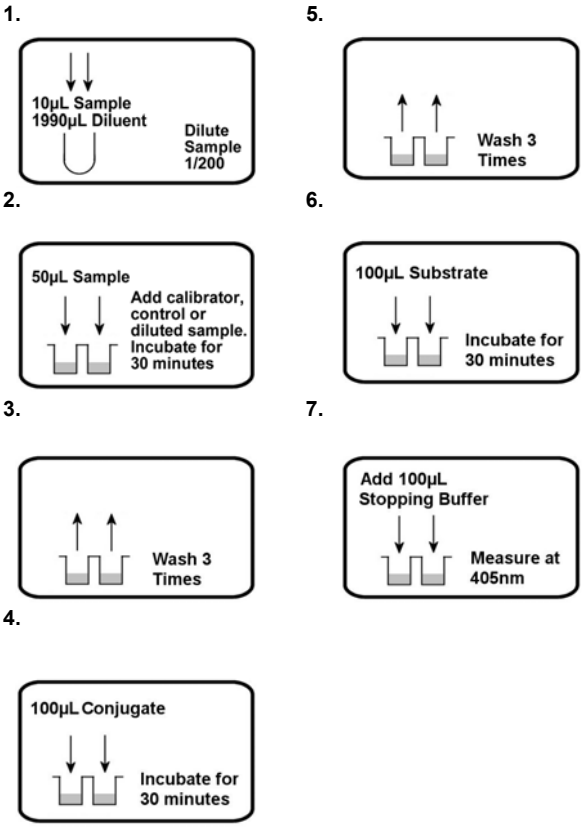
Therefore, the calibrators should be handled using the same safety precautions employed when handling any potentially infectious material.

Used calibrators, controls, samples, pipette tips and plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

ABTS™ (2, 2'-azino-bis (3-ethylbenzothiazoline-6 sulphonic) acid) is a trademark of Roche Diagnostics.

Safety data sheets are available on request

Test Procedure



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