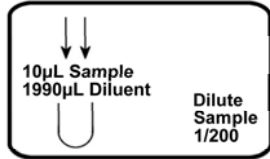
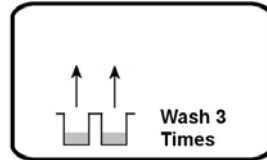


Test Procedure

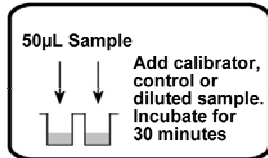
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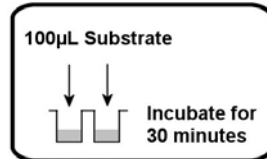
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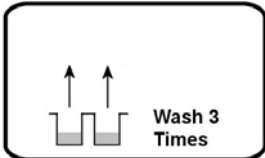
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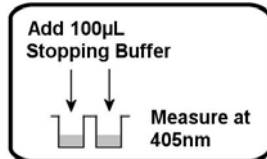
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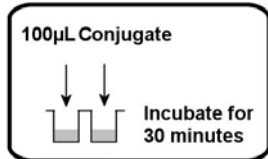
3.



7.



4.



AUTOZYME™ TAB

Anti-Tg and Anti-TPO antibodies

Anti-Tg: Z2196
Anti-TPO: Z2396

Instructions for Use

A5272.08
Apr '05



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Table of Contents

Intended Use	3
Background	3
Principle	4
Kit Contents	5
Storage	6
Sample Handling	6
Additional Reagents and Equipment Required	6
Procedural Precautions	6
Assay Procedure	7
Calculation of Results	9
Quality Control	9
Performance Data	9
Safety Precautions	11
Bibliography	12

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 is a range of enzyme immunoassays (EIAs) for the screening and detection of auto-antibodies against human thyroglobulin (Tg) and thyroid peroxidase (TPO) in human serum. The assays are 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 preparations:

Anti-Tg - NIBSC 65/93 (1st IRP)

Anti-TPO - NIBSC 66/387

AUTOZYME™ TAB 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 Tg and TPO antigens. TPO has been identified as the specific antigenic determinant of the thyroid microsomal antigen³.

The presence of auto-antibodies to thyroid antigens correlates with the degree of lymphocytic infiltration of the thyroid gland⁴, the hallmark of Hashimoto's thyroiditis. Circulating auto-antibodies to Tg and 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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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.

It is reported^{5,6} that low levels of autoimmune antibodies predict at-risk pregnancy. Furthermore, a report⁷ 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-Tg uses human thyroglobulin. AUTOZYME™ TAB Anti-TPO uses recombinant human thyroid peroxidase which does not contain contaminating thyroglobulin and/or mitochondria found in other microsomal antigen preparations. Both of these assays are in an EIA test format.

3. Principle

AUTOZYME™ TAB 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 Tg and TPO wells are provided coated with purified antigen (human thyroglobulin and recombinant TPO respectively). When calibrators, controls or diluted sera are added, any anti-thyroid 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-Tg (IU/mL)	Anti-TPO (IU/mL)
1	0	0
2	100	25
3	300	100
4	750	250
5	3000	1000
6	7500	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

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-Tg antibodies	20 IU/mL
anti-TPO antibodies	1 IU/mL

c. Reference values:

AUTOZYME™ TAB was used to determine the anti-Tg and 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-Tg antibodies

Normal range	≤ 180 IU/mL
Borderline	181 - 230 IU/mL
Positive	≥ 231 IU/mL

Anti-TPO antibodies

Normal range	≤ 50 IU/mL
Borderline	51 - 99 IU/mL
Positive	≥ 100 IU/mL

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

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 and anti-Tg 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-Tg Antibodies		Anti-TPO Antibodies	
	IU/mL	CV%	IU/mL	CV%
Intra-assay (n=20)				
Sample 1	5281	12.9	500.3	8.3
Sample 2	2496	9.8	902.9	7.9
Sample 3	138.0	7.2	259.7	10.5
Inter-assay (n = 12)				
Sample 1	4607	9.7	565.2	18.1
Sample 2	2494	9.0	932.3	17.2
Sample 3	130.5	9.6	231.8	9.6

9

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 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 Required

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 carryover.

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). AUTOZYME™ TAB has been designed so that AUTOZYME™ Anti-Tg and Anti-TPO antibodies assays can be run simultaneously. The wash buffer, sample diluent substrate and stop solutions are common.

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

9. 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 samples be tested in 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.