AB Diagnostic Systems

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CE

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

INSTRUCTIONS FOR USE
abia T4 total
Enzyme immunoassay for the quantitative determination of total thyroxine (T4) concentration in human serum

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

I. INTENDED USE

The abia T4 total kit is intended for the quantitative determination of total thyroxine 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

L-Thyroxine (T4) is a hormone that is synthesized and stored in the thyroid gland. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of T4 is reversibly bound to three plasma proteins in blood – thyroxine binding globulin (TBG) binds 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%. Approximately 0.03% of T4 is in the free, unbound state in blood at any one time. Diseases affecting thyroid function may present a wide array of confusing symptoms. Measurement of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in patients. Increased levels of T4 have been found in hyper-thyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease), and with some genetic abnormalities.

III. PRINCIPLE OF THE TEST

The abia T4 total is a one-step immunoassay to determine the presence of total thyroxine (total T4) in human serum using competitive microplate enzyme immunoassay.

Plates are coated with anti-T4 antibodies. Serum reference, patient specimen, or control is first added to microplate well. Enzyme-T4 conjugate is added. Thyroxine present in the sample competes with Enzyme-thyroxine conjugate for binding with anti-T4 coated microplate to form an antigen-antibody complex.

Unbound conjugate is removed by washing. The enzyme activity in the antibody-bound fraction is inversely proportional to the native thyroxine concentration. The enzyme activity is revealed by a color change in TMB-Substrate solution.

IV. CONTENT OF THE KIT abia T4 total

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
T4 antibody coated microtiter wells	Polystyrene stripped 96-well (breakable wells) plate coated with anti-thyroxine monoclonal antibodies. Once opened, microtiter wells should be stored at 2-8 °C during shelf-life of the kit.	1 plate
Conjugate	Thyroxine, conjugated with HRP enzyme in a protein-stabilized matrix. Pink transparent liquid. Preserving agent: 0.1% ProClin 300, 0.004% gentamycin sulfate. Once opened, Conjugate should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 12.0 ml
Calibrator 0 Calibrator 1 Calibrator 2 Calibrator 3 Calibrator 4 Calibrator 5	Six vials of human serum based reference calibrators for total thyroxine. The thyroxine concentration levels in Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis.* Transparent or slightly opalescent liquids, colorless, or pale yellow. Preserving agent: 0.1% ProClin 300, 0.01% thimerosal, 0.1% phenol. Once opened, Calibrators should be used within two months. Store at 2-8 °C in tightly sealed vials.	6 vials 0.5 ml
Control Serum	Human serum with a defined quantity of T4. The thyroxine concentration level in Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent liquid, colorless, or pale yellow. Preserving agent: 0.1% ProClin 300, 0.01% thimerosal, 0.1% phenol. Once opened, Control Serum should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 0.5 ml
T4 buffer ANS	Buffer, binding protein inhibitors. Transparent olive-brown colored liquid. Preserving agent: 0.05% ProClin 300. Once opened, buffer should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 12.0 ml
Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent liquid, colorless, or pale yellow. Once opened, Washing Solution should be stored at 2-8 °C until the expiry date of the kit.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine in citric buffer solution, containing H_2O_2. Transparent colorless liquid. Once opened, TMB-Substrate should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulphuric acid solution. Transparent colorless liquid. Once opened, Stopping Reagent should be stored at 2-8 °C until the expiry date of the kit.	1 vial 25.0 ml

* Nominal values of Calibrators are traceable to a collection of serum samples certified using a chemiluminescence immunoassay analyzer in accordance with EN ISO 17511:2003 In vitro diagnostic medical devices – Measurement of quantities in biological samples – Metrological traceability of values assigned to calibrators and control materials.

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 laboratory should be 18-25 °C.
- Inspect the contents of the box: check the vials and labels integrity. If labels are lost 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.
- Never use the same container to distribute conjugate and other solutions.
- 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 substrate solutions.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Do not let the wells dry once the assay has been started.
- 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.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use high quality 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".
- 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).
- 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, Calibrators 0-5, Control Serum, T4 buffer ANS contain 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 (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collection of blood samples should be implemented according to the current practices. Serum only may be used. Separate serum from blood cells 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 results. Do not heat the samples. The blood should be collected in plain redtop venipuncture tube without additives and gel barrier.

Samples can be stored at 2-8 °C not more than for 72 hours; they may be deep-frozen at -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 and which were preserved by sodium azide must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **T4 antibody 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.
- **Calibrators 0-5;**
- **Control Serum;**
- **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. Thoroughly mix the solution. The prepared Working Washing Solution is stable for 14 days at room temperature or for 28 days at 2-8 °C.
- **Working conjugate solution.** Working conjugate solution must be prepared before usage. To make working conjugate solution take equal amounts of conjugate and T4 buffer ANS in a separate vial. Thoroughly mix the working conjugate solution. For example mix 2.0 ml of conjugate and 2.0 ml of T4 buffer ANS for 16 wells (a slight excess of solution is made). This reagent may not be stored.

X. TEST PROCEDURE

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

1. To the wells add 25 µl of Calibrators and Control Serum in duplicate. Leave two wells for OD control of TMB-Substrate.

2. To the rest of the wells, add 25 µl of samples in duplicate. Pipetting of samples should not extend beyond ten (10) minutes.

3. Add 200 µl of working conjugate solution to all wells except for the wells for OD control of TMB-Substrate.

4. Swirl the microplate gently for 30 seconds after adding of samples and working conjugate solution to mix, cover the strips with a lid or a protective film and incubate for 90 minutes at room temperature (here 20-25 °C).

5. Aspirate the contents of the wells into the container with disinfecting solution. Wash the plate 5 times with 300 µl of Working Washing Solution per well and remove Working Washing Solution using a washer into the container with disinfecting solution. Tap the plate firmly against absorbent paper to ensure that it is dry – the residual volume must be lower than 10 µl (the use of a washer is recommended). Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.

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

7. Incubate for 15-20 minutes at room temperature in the dark.

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

9. Read the absorbance on the microplate reader at 450 nm within 20 minutes after stopping reaction.

Scheme of the assay is represented in Annex.

Spectrophotometric verification of reagent pipetting

The presence of working conjugate solution + sample in the well can be verified by automatic reading at 540 (550) nm. Each well containing sample and working conjugate solution must have an OD higher than 0.500.

10. 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 XIV). 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 graph paper with the mean absorbance on Y axis and the calibrator concentration on the X axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean absorbance values for each specimen.
4. Read the value of T4 concentration in nmol/l in the unknowns directly off the calibration curve.

Typical tabulated data

Calibrator	OD 1	OD 2	Mean OD	Value (nmol/l)
0	3.138	3.092	3.115	0
1	2.089	2.134	2.111	11
2	0.969	0.990	0.979	51
3	0.587	0.603	0.595	94
4	0.323	0.327	0.325	190
5	0.182	0.185	0.183	400
unknown	0.582	0.707	0.644	88.8

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 ≤ 0.2.
2. The absorbance (OD) of Calibrator 0 should be ≥ 1.3.
3. Calculated value of Control Serum should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia T4 total

1. Sensitivity

The lower detection limit does not exceed 5.0 nmol/l. The sensitivity was ascertained by determining the variability of the 0 nmol/l serum calibrator and using the 2 SD (95% certainty) statistics to calculate the minimum dose.

2. Specificity

The following compounds were tested for cross-reactivity with abia T4 total.

Substance	The added quantity of cross reagent, ng/ml	Cross reactivity, %	
		T4 total Low	T4 total High
L-Thyroxine	50	100	100
Tetraiodothyroacetic acid	1000	0.75	0.03
Triiodo-L-thyronine	1000	0.71	0.12
Diiodo-L-thyronine	200000	0.004	0.001

3. Precision

The repeatability of the abia T4 total was determined by analyses on three different levels of pool control sera. Three serum pools were assayed in duplicate twice per day for 20 days (80 total measurements). The number (N), mean values (Mean), standard deviation (SD) and coefficient of variation (CV) for each of these control sera are presented in Table.

Precision of the abia T4 total

Sample Pool #	Mean Total T4 Conc. (nmol/l)	Repeatability		Between-run Precision		Within-device Precision	
		SD	CV%	SD	CV%	SD	CV%
1	73.3	3.3	4.4	2.3	3.2	4.0	5.5
2	144.1	7.9	5.5	7.0	4.8	10.5	7.3
3	263.9	10.8	4.1	15.5	5.9	18.9	7.2

4. Expected normal Value

A normal range of 59.0 to 153.0 nmol/l was obtained by testing serum specimens from euthyroid adult 305 individuals. It is strongly recommended that each laboratory should determine its own normal range values.

Unit Conversion Calculator: nmol/l x 0.777 = ng/ml; ng/ml x 1.287 = nmol/l.

5. Accuracy

The abia T4 total test system was compared with a Chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 336.

The least square regression equation and correlation coefficient were computed for abia T4 total in comparison with the reference method. The least square regression analysis was y = 0.996(x) + 4.89 with correlation coefficient 0.95.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the determination of thyroxine in human serum. The kit is not calibrated for the T4 determination in saliva, plasma or other specimens of human or animal origin.
2. Highly lipemic, hemolyzed or grossly contaminated specimens should not be used.
3. It is important that the time of reaction in each well is held constant for reproducible 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 results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has to potential of causing interferences in immunological tests. For diagnostic purposes, results should be used in conjunction with other data; eg., symptoms, results of other thyroid tests, clinical impressions, etc.
7. Serum total T4 values may be changed under conditions such as pregnancy.
8. A decrease in total thyroxine values is found with protein wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions which affect total thyroxine values has been compiled by the Journal of the American Association of Clinical Chemists.
9. In rare conditions associated with extreme variations in albumin binding capacity for T4 – such as familial dysalbuminemic hyperthyroxinemia – direct assessment of T4 may be misleading.
10. Circulating antibodies to T4 and hormone binding inhibitors may interfere in the performance of the assay.
11. Performance of this test has not been established with neonatal specimens.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging.

Keep in dark dry place at 2-8 °C. Freezing is prohibited.

Transportation should be done at 2-8 °C. Transportation at 9-20 °C is allowed not more than during ten (10) days.

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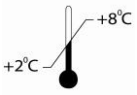
XV. REFERENCES

1. Sterling L., "Diagnosis and treatment of Thyroid Disease", CRC Press, 19-51 (1975).

2. Nelson J.C. and Wilcox, RB. "Analytical performance of Free and Total thyroxine assay". Clin. Chem. Vol. 42, 146-154 (1996).

XVI. EXPLANATION OF SYMBOLS

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

Annex

Scheme of the assay

1	**Add**	25 µl of Calibrators, Control Serum in duplicates; 25 µl of samples in duplicates; two wells for OD control of TMB-Substrate
2	**Add**	200 µl of working conjugate solution into all wells except for the wells for OD control of TMB-Substrate
3	**Mix**	30 seconds
4	**Incubate**	90 min, at 20-25 °C
5	**Wash the plate**	**Working Washing Solution, 300 µl, 5 times**
6	**Add**	100 µl of TMB-Substrate into all wells
7	**Incubate**	15-20 min, at room temperature in a dark place
8	**Add**	150 µl of Stopping Reagent into all wells
9	**Mix**	5-10 seconds
10	**Read the optical density**	450 nm

nmol/l x 0.777 = ng/ml ng/ml x 1.287 = nmol/l