AB Diagnostic Systems

REF **DK.033.01.8** Σ **96** IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia T3 total
Enzyme immunoassay for the quantitative determination of total triiodothyronine (T3) 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 T3 total kit is intended for the quantitative determination of total triiodothyronine 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

Triiodothyronine (T3) is the principal thyroid hormone with a molecular weight of 650 Daltons. T3 circulates in the blood as an equilibrium mixture of free and protein bound hormone. T3 is bound to thyroxine-binding globulin (TBG), prealbumin and albumin. However, only the free (unbound) portion of triiodothyronine is responsible for the biological action. The total triiodothyronine concentration can help in the assessment of thyroid status.

III. PRINCIPLE OF THE TEST

The abia T3 total is a one-step immunoasay to determine the presence of total triiodothyronine (total T3) in human serum using competitive microplate enzyme immunoassay.

Plates are coated with anti-T3 antibodies. Serum reference, patient specimens or control serum is first added to microplate well. Enzyme-T3 conjugate is added. A competition reaction results between the enzyme-triiodothyronine conjugate and a serum containing the native total triiodothyronine for a limited number of antibody combining sites are immobilized on the well.

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

IV. CONTENT OF THE KIT abia T3 total

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
T3 antibody coated microtiter wells	Polystyrene stripped 96-well (breakable wells) plate coated with anti-triiodothyronine monoclonal antibodies. Once opened, microtiter wells may be stored at 2-8 °C during shelf-life of the kit.	1 plate
Conjugate	Triiodothyronine, conjugated with HRP enzyme in a protein-stabilized matrix. Pink transparent or opalescent liquid. Preserving agent: 0.1% ProClin 300. 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	Five vials of human serum based reference calibrators for total triiodothyronine. The total triiodothyronine concentration levels in the Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis.* Transparent or slightly opalescent liquids, pale yellow. Preserving agents: 0.1% ProClin 300, 0.01% thimerosal, 0.1% phenol. Once opened, the calibrators should be used within two months. Store at 2-8 °C in tightly sealed vials.	5 vials 0.5 ml
Control Serum	Human serum with a defined quantity of total T3. The total triiodothyronine concentration level in the Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent liquid, pale yellow. Preserving agents: 0.1% ProClin 300, 0.01% thimerosal, 0.1% phenol. Once opened, the control serum should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 0.5 ml
T3-8ANS	Buffer with binding protein inhibitor. Transparent olive-brown liquid. Preserving agent: 0.05% ProClin 300. Once opened, the T3-8ANS should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 6.0 ml
Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent liquid, colorless, or pale yellow. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (TMB) in citric buffer solution, containing H_2O_2. Transparent colorless liquid. Once opened, the 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. Store at 2-8 °C until expiration date in a tightly sealed vial.	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 lab should be 18-25 °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 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.
- Never use the same container to distribute conjugate and color development solution.
- 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 has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious 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 0-4, Control Serum, T3-8ANS 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.

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:

- **T3 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 0-4;**
- **Control Serum;**
- **TMB-Substrate;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Conjugate-T3-8ANS working solution.** Mix 1 volume of T3-8ANS and 2 volumes of Conjugate in a suitable container (for example, 0.5 ml of T3-8ANS + 1.0 ml of Conjugate for 1 strip). The mixed reagent is not subject to storage.
- **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.

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 150 µl of Conjugate-T3-8ANS working 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 Conjugate-T3-8ANS working 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 Conjugate-T3-8ANS working solution + sample in the well can be verified by automatic reading at 540 (550) nm. Each well containing sample and Conjugate-T3-8ANS working 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 calibration concentration on the X axis.
3. Calculate the mean absorbance values for each specimen.
4. Read the value of total T3 concentration in ng/ml in the unknowns directly off the calibration curve. If immunoassay software is being used, a 4-parameter curve is recommended.

Typical tabulated data

Calibrator	OD 1	OD 2	Mean OD	Value (ng/ml)
0	2.799	2.777	2.788	0
1	2.318	2.324	2.321	0.5
2	1.032	1.002	1.017	2.5
3	0.450	0.468	0.459	5.2
4	0.229	0.215	0.222	8.8
unknown	1.712	1.716	1.714	1.2

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 not be more than 0.2 at 450 nm.
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 T3 total

1. Sensitivity

The lower detection limit is 0.2 ng/ml. The sensitivity was calculated by determining the variability of the 0 ng/ml serum calibrator and using the 2 SD (95% certainty) statistics.

2. Specificity

The following compounds were tested for cross-reactivity with abia T3 total

Substance	Cross reactivity, %
Triiodothyronine	100
L-Thyroxine	0.0001
Diiodothyronine	0.0001
Tetraiodothyroacetat	0.0001

3. Intra-Assay Precision

Two samples were assayed 9 times each on the same calibrator curve. The results are tabulated below:

Sample	Mean	SD	CV %
1	2.2	0.0983	4.5
2	4.2	0.1452	3.5

4. Inter-Assay Precision

Two samples were assayed 2 times 3 runs a day. The results are tabulated below:

Sample	Mean	SD	CV %
1	2.3	0.0957	4.2
2	4.2	0.2381	5.9

5. Expected normal Value

A normal range of 0.56 to 1.88 ng/ml was obtained by testing serum specimens from 220 individuals determined as normal by abia T4 total and abia TSH assays. It is strongly recommended that each laboratory should determine its own normal range values.

Unit Conversion Calculator: nmol/l x 0.651 = ng/ml; ng/ml x 1.536 = nmol/l.

6. Accuracy

The abia T3 total kit was compared with a reference Chemiluminescent microparticle immunoassay. The total number of specimens was 210. The least square regression equation and correlation coefficient were computed for abia T3 total in comparison with the reference method. The least square regression equation is $y = 0.98(x) + 0.14$. Correlation coefficient is 0.96.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the determination of total triiodothyronine in human serum. The kit is not calibrated for the total T3 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; e.g. symptoms, results of other thyroid tests, clinical impressions, etc.
7. Serum total T3 values may be changed under conditions such as pregnancy.
8. A decrease in total triiodothyronine values is found with protein wasting diseases, certain liver diseases and administration of diphenylhydantoin or salicylates. A table of interfering drugs and conditions which affect total Triiodothyronine values has been compiled by the Journal of the American Association of Clinical Chemists.
9. Circulating antibodies to T3 and hormon binding inhibitors may interfere in the performance of the assay.
10. 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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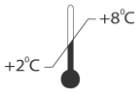
XV. REFERENCES

1. Young D.S., Pestaner L.C., Clin. Chem., Vol.21, 3660 (1975).
2. Kozwich D., Davis G., Sockol C., Clin. Chem., Vol. 37, 1040 (1991).
3. Ekins, “Total hormon assay”. Nuclear Medicine Communications, 14, 676-688 (1993).

XVI. EXPLANATION OF SYMBOLS

	For *in vitro* diagnostic use		Expiry date CCYY-MM-DD
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM		Symbol “exclamation mark”
	Catalog number		Symbol “corrosion”
	Sufficient for	**Warning! Danger!**	Signal words
	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**	150 µl of Conjugate-T3-8ANS working 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 0.651 = ng/ml

ng/ml x 1.536 = nmol/l