AB Diagnostic Systems

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CE

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

INSTRUCTIONS FOR USE
abia fT4
Enzyme immunoassay for the quantitative determination of free thyroxine (fT4) 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 fT4 kit is intended for the quantitative determination of free thyroxine concentration in human serum by a microplate enzyme immunoassay.

The assay is recommended for laboratory diagnosis of thyroid diseases.

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

Thyroxine, the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. The main carrier is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of thyroxine is responsible for the biological action. Futher, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total thyroxine level changes so that the free thyroxine concentration remains constant. Thus, measurements of free thyroxine concentrations correlate better with clinical status than total thyroxine level.

The free thyroxine concentration can help in the assessment of thyroid status.

III. PRINCIPLE OF THE TEST

The abia fT4 is a one-step immunoasay to determine the presence of free thyroxine (free T4) in human serum using competitive microplate enzyme immunoassay.

Plates are coated with anti-T4 antibodies. Serum reference, patient speciment, or control is first added to microplate well. Enzyme-T4 conjugate is added. Free 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 free thyroxine concentration. The enzyme activity is revealed by a color change in TMB-Substrate solution.

IV. CONTENT OF THE KIT abia fT4

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-T4 coated microtiter wells	Polystyrene stripped 96-well plate (breakable wells) coated with anti-thyroxine monoclonal antibodies. Once opened, microwell strips 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: 0.004% gentamycin sulfate, 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 Calibrator 5	Six vials of human serum based reference calibrators of free thyroxine. The free 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, pale yellow. Preserving agent: 0.1% ProClin 300, 0.1% phenol. Once opened, Calibrators should be used within two months. Store at 2-8 °C in a tightly sealed vials.	6 vials 0.5 ml
Control Serum	Human serum with a defined quantity of free thyroxine. The free 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, pale yellow. Preserving agent: 0.1% ProClin 300, 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
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. 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 (TMB) in citric buffer solution, containing H_2O_2. Transparent colorless liquid, coloration is possible. 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 sulfuric 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
Protective films for EIA plates		2
Disposable tips		16

Disposable plastic dishes for liquid reagents	2
Polyethylene bag with a Zip-Lock	1
Calibration Curve Form	1
Instructions for use	1

***** Nominal values of Calibrators are traceable to a collection of serum samples certified using a chemiluminescence immunoassay in accordance with EN ISO 17511:2021 In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples.

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 color development solution.
- 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 Calibrators and Control Serum has 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 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 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 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.

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 and which were preserved by sodium azide, thimerosal must not be analyzed.

Samples with high bilirubin levels should not be assayed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-T4 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 0-5;**
- **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. The prepared Working Washing Solution is stable for 14 days at room temperature or for 28 days at 2-8 °C.

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		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1 well**
Working Washing Solution	Washing Solution (×25), ml	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	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. 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 100 µl of Conjugate 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 to mix, cover the strips with 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 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 20-30 minutes at room temperature in the dark.

8. Stop the reaction by adding 150 µl of Stopping Reagent to the wells, shake the strips for 20 seconds and read the results. The time between stopping the reaction and measuring OD should not exceed 20 min.

9. Read the absorbance on the microplate reader at 450 nm.

Scheme of the assay is represented in Annex.

Spectrophotometric verification of reagent pipetting

The presence of Conjugate + sample in the well can be verified by automatic reading at 492 nm. Each well containing sample and Conjugate must have an OD higher than 0.150.

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 free T4 concentration in pmol/l in the unknowns directly off the calibration curve.

Typical tabulated data

Calibrator	OD 1	OD 2	Mean OD	Value (pmol/l)
0	2.825	2.824	2.825	0
1	2.533	2.445	2.489	5
2	2.088	2.061	2.075	10
3	1.325	1.279	1.302	21
4	0.770	0.760	0.765	40
5	0.143	0.133	0.138	100
Unknown	1.716	1.720	1.718	14.5

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 fT4

1. Analytical sensitivity:

- The lowest measurable concentration of free T4 in serum is 1.0 pmol/l;
- LoB (limit of blank) – 0.83 pmol/l;
- LoD (limit of detection) – 1.072 pmol/l;
- LoQ (limit of quantitation) – 12.0 pmol/l.

2. Analytical specificity:

- endogenous interference substances: no interference observed in the samples containing albumin up to 40 mg/ml, bilirubin up to 0.065 mg/ml, hemoglobin up to 1.0 mg/ml, lipids up to 5.0 mg/ml.

- exogenous interference substances: no interference observed in the samples containing sodium salicylate up to 2.5 mg/ml, heparin up to 330 U/ml, dexamethasone up to 0.12 mg/ml, amiodarone up to 0.042 mg/ml. The administration of furosemide affects the results of testing serum for free T4 levels.

- no cross-reactivity with structurally related compounds was observed: with triiodothyroacetate up to 20000 pg/ml, diiodothyronine up to 20000 pg/ml; triiodothyronine up to 1000000 pg/ml.

3. Reproducibility and repeatability

The studies of within-plate reproducibility (repeatability), between-day precision, within-laboratory precision, reproducibility between laboratories (devices) and operators, between-lot precision and between-plate of one lot were carried out in accordance with CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (scheme 20-2-2) and in accordance with CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-Third Edition (scheme 3-5-5).

Coefficient of variation did not exceed 8 %.

4. Accuracy

The assay accuracy was determined by a comparative assessment of serum of hypo- and hyperthyroidism patients and of individuals with euthyroidism in the abia fT4 assay and in a reference kit. $R^2 = 0.9628$ ($R^2 \geq 0.95$).

5. Reference intervals

Free T4 levels were measured in serum samples drawn from 9 to 11 hours, from 240 healthy individuals aged 21 to 50. The average free T4 concentration was 15.6 pmol/l (normal range 9.0 to 22.2 pmol/l).

During pregnancy, free T4 levels can differ from the reference range. It is recommended that each laboratory should determine free thyroxine levels corresponding to the range of normal values for a certain territory using the assay.

6. Analytical range

Analytical measurement range is 0-130 pmol/l.

XIII. LIMITS OF THE TEST

- The kit is not intended for the determination of thyroxine in saliva, plasma and other samples of human or animal origin.
- The decision on the clinical diagnosis must not be based solely on the results of this assay. For instance, the presence of heterophile antibodies in the patients contacting with animals might affect the enzyme immunoassay results. The assay results must be used in conjunction with other clinical information for the diagnostic purposes: symptoms, overall clinical presentation, results of other assays.
- Some pharmaceuticals affecting the T4 binding to the thyroid hormone transport proteins or their metabolism makes it difficult to interpret the results of testing for free T4. Major non-thyroidal diseases can also present challenges in assessing the results of free T4 determination. In such cases, a testing for the determination of thyroid-stimulating hormone (TSH) is recommended.
- In rare cases, such as familial dysalbuminemic hyperthyroxinemia, a direct T4 testing can give false results.
- Circulating antibodies to T4 can affect the assay results.

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 9-25 °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, R.B. “Analytical perfomance of Free and Total thyroxine assay”. Clin. Chem. Vol. 42, 146-154 (1996).

3. Midgeley J. “Direct and Indirect Free Thyroxine Assay Methods in Theory and Practice”, Clin. Chem., Vol. 47, 1353-1363 (2001).

4. Gong Y., Hoffman B.R. “Free thyroxine reference interval in each trimester of pregnancy determined with the Roche Modular E-170 electrochemiluminescent immunoassay”, Clin. Biochem, Vol. 41(10-11): 902-6 (2008).

5. Lee R.H., Spencer C.A., Mestman J.H. “Free T4 immunoassays are flawed during pregnancy”, Am. J. Obstet. Gynecol. 200(3):260.e1-6 (2009).

6. Stricker R., Echenard M., Eberhart R., Maud R. “Evaluation of maternal thyroid function during pregnancy: the importance of using gestational age-specific reference intervals”, European Journal of Endocrinology, Vol. 157: 509-514 (2007).

XVI. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)		
	Manufacturer	+2°C +8°C	Storage temperature limitation
	Date of manufacture CCYY-MM		Consult Instruction for use
	Expiry date CCYY-MM-DD	IVD	For in vitro diagnostic use
LOT	Batch code	Σ	Sufficient for
REF	Catalog number	!	Symbol “exclamation mark”
	Do not use if package is damaged	**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**	25 µl of Calibrators, Control Serum in duplicates; 25 µl of samples in duplicates two wells for OD control of TMB-Substrate
2	**Add**	100 µl of Conjugate into all wells, except for the wells for OD control of TMB-Substrate
3	**Mix**	30 seconds
4	**Incubate**	60 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**	20-30 min, at room temperature in a dark place
8	**Add**	150 µl of Stopping Reagent into all wells
9	**Mix**	20 seconds
10	**Read the optical density**	450 nm

pmol/l x 0.777= pg/ml

pg/ml x 1.287 = pmol/l