AB Diagnostic Systems

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

INSTRUCTIONS FOR USE
abia fT3
Enzyme immunoassay for the quantitative determination of free triiodothyronine (fT3) concentration in human serum

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 assays (one breakable wells) including controls; the kit is intended for manual testing with a possibility of fractional (one well) use of the kit or use of the kit on open type automated analyzer for enzyme immunoassay.

I. INTENDED USE

The abia fT3 kit is intended for the quantitative determination of free 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, 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 triiodothyronine 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 triiodothyronine level changes so that the free triiodothyronine concentration remains constant. Thus, measurements of free triiodothyronine concentrations correlate better with clinical status than total triiodothyronine level.

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

III. PRINCIPLE OF THE TEST

The abia fT3 is a one-step immunoasay to determine the presence of free triiodothyronine (free T3) in human serum using competitive microplate enzyme immunoassay.

Plates are coated with anti-T3 antibodies. Serum reference, patient specimen, or control is first added to microplate well. Enzyme-T3 conjugate is added. T3 present in the sample competes with Enzyme-T3 conjugate for binding with anti-T3 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 triiodothyronine concentration. The enzyme activity is revealed by a color change in TMB-Substrate solution.

IV. CONTENT OF THE KIT abia fT3

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
T3 antibody Coated Strips	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal antibodies to triiodothyronine. Store at 2-8 °C until expiration date of the kit.	1 plate
Conjugate	Triiodothyronine, conjugated with HRP enzyme in a protein-stabilized matrix. Transparent or opalescent pink color liquid. Preserving agent: 0.1% ProClin 300. Store at 2-8 °C until expiration date 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 free triiodothyronine. The free Triiodothyronine 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. Store at 2-8 °C until expiration date in tightly sealed vials.	5 vials 0.5 ml
Control Serum	Human serum with a defined quantity of free T3. The free Triiodothyronine concentration level in Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Transparent or opalescent liquid, pale yellow. Preserving agent: 0.1% ProClin 300, 0.1% phenol. Store at 2-8 °C until expiration date 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. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric acid buffer, containing H_2O_2 (0.01%). Transparent colorless liquid. Store at 2-8 °C until expiration date in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulfuric 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 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.

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;
- a self-sealing plastic bag.

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the laboratory 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 substrate solutions.
- 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 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).
- Certain reagents contain biological material of animal origin.
- 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 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.
- Microplate shaker (at room temperature), 500-800 rpm.
- 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 must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **T3 antibody Coated Strips.** 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 0-4;**
- **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.

Number of strips to be used		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
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
	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

X. TEST PROCEDURE

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

Step	**Test procedure**
1	To the wells add 25 µl of Calibrators and Control Serum in duplicate. Leave two wells for OD control of TMB-Substrate (blank).
2	To the rest of the wells, add 25 µl of samples in duplicate. **The time of adding samples should not exceed 10 min.**
3	To all wells, except for those with TMB-Substrate control, add 100 µl of Conjugate.
4	Cover the plate with a lid or a protective film and incubate on a microplate shaker (approximately 500-800 rpm) for 60 minutes at room temperature.
5	Aspirate the contents of the wells into the container with disinfecting solution. Wash the plate **5 times** with the working Washing Solution. For this, pipette the working Washing Solution up to the top of the wells **(not less than 300 µl per well)**. Then aspirate the liquid to a disinfectant container. If necessary, knock the plate out onto filter paper folded several times to remove the

	residual moisture. *It is recommended to use automated microtiter washer. Inadequate washing may adversely affect the accuracy of the assay.*
6	Add 100 µl of TMB-Substrate into each well.
7	Incubate for 20-30 minutes at room temperature in a dark place.
8	Stop the reaction by adding 150 µl of Stopping Reagent to the wells, shake the strips for 5-10 seconds and read the results. The time between stopping the reaction and measuring OD should not exceed 20 min. Read the plate on 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 540 (550) nm. Each well containing sample and Conjugate must have an OD higher than 0.500.

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 XV). 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 optical density of each Calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "blank" from the mean absorbance values of the Calibrators, Control Serum and serum samples.
4. Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.

Typical Tabulated Data

Calibrator	OD 1	OD 2	Mean OD	Value (pg/ml)
0	2.624	2.578	2.601	0
1	1.985	1.976	1.981	1.5
2	1.397	1.423	1.410	4.5
3	0.889	0.905	0.897	10
4	0.574	0.594	0.584	20
unknown	1.690	1.712	1.701	2.6

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 not be more than 0.2 at 450 nm.
2. The absorbance (OD) of **Calibrator 0** should be ≥ 1.3 at 450 nm.
3. Calculated value of **Control Serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF abia fT3

1. Assay Dynamic Range

The range of the assay is between 0-20 pg/ml.

2. Analytical sensitivity

The lower detection limit is 0.3 pg/ml. The sensitivity was calculated by determining the variability of the 0 pg/ml serum calibrator and using the 2 SD (95% certainty) statistics.

3. Specificity (cross-reactivity)

The following compounds were tested for cross-reactivity with abia fT3.

Substance	Cross reactivity,%
Triiodothyronine	100
L-Thyroxine	0.0006
Diiodothyronine	0.0001
Tetraiodothyroacetat	0.00001

4. Intra-Assay Precision

Two samples were assayed 10 times each on the same calibrator curve. The results are tabulated below:

Sample	Mean	SD	CV,%
1	2.34	0.102	4.3
2	6.09	0.135	2.2

5. Inter-Assay Precision

Two samples were assayed 4 times on the different calibrator curves. The results are tabulated below:

Sample	Mean	SD	CV,%
1	2.3	0.118	5.1
2	5.97	0.217	3.6

6. Expected normal Value

A normal range of 1.2 to 4.2 pg/ml was obtained by testing serum specimens from 250 individuals determined as normal by abia fT3 and abia TSH assays. It is strongly recommended that each laboratory should determine its own normal range values.

Unit Conversion Calculator: pmol/l x 0.651 = pg/ml; pg/ml x 1.536 = pmol/l.

7. Accuracy

The abia fT3 test-system was compared with a Chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 726. The least square regression equation and correlation coefficient were computed for abia fT3 in comparison with the reference method. The least square regression analysis was y = 1.51x-1.96 with correlation coefficient 0.93.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the determination of free triiodothyronine in human serum. This test is not calibrated for the free T3 determination in saliva, plasma or other specimens of human or animal origin.
2. Any improper handing of samples or modification of this test might influence the results.
3. Highly lipemic, hemolyzed or grossly contaminated specimens should not be used.
4. It is important that the time of reaction in each well is held constant for reproducible results.
5. If more than 1 plate is used, it is recommended to repeat the dose response curve.
6. Do not touch the bottom of the wells.
7. 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.
8. Serum free T3 values may be changed under conditions such as pregnancy.
9. The interpretation of free T3 is complicated by a variety of drugs that can affect the binding of T3 to the thyroid hormon carrier proteins. In severe non-thyroidal illness (NTI) the assessment of thyroid becomes especially difficult. Since the patients in this category may suffer from concominant primary hypothyroidism or from compensatory secondary hypothyroidism. In cases like these a sensitive TSH evaluation of the patient may be recommended. Please use abia TSH, AB Diagnostic Systems GmbH.
10. In rare conditions associated with extreme variations in albumin binding capacity for T3 – such as familial dysalbuminemic hyperthyroxinemia – direct assessment of free T3 may be misleading.
11. Circulating antibodies to T3 and hormon binding inhibitors may interfere in the performance of the assay.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

- **Expiry date is indicated on the packaging.** Storage and transportation conditions for the kit, conditions and terms of storage for working solutions and unused reagents are specified in Table 2.

- Transportation should be done at specified temperature in accordance with established transportation regulations. Kits transported at improper temperature cannot be used.
- Kits stored improperly cannot be used.

Table 2

1	**Storage conditions**		
	Keep in a dark dry place at 2-8 °C. Freezing is prohibited. Do not use expired kits.		
2	**Transportation conditions**		
	at 2-8 °C		
	at 9-20 °C	not more than during ten (10) days	
3	**Conditions and terms of storage for working solutions** Keep in a dark dry place and in a chemically neutral vial		
	Working Washing Solution	at 2-8 °C	For up to 28 days
		at 18-24 °C	For up to 14 days
4	**Conditions and terms of storage of unused reagents after opening**		
	Keep in a dark dry place at 2-8 °C.		
	T3 antibody Coated Strips	Place the unused strips/wells back into the bag, reseal the foil-lined package in Zip-Lock plastic bag. Do not remove desiccant.	Until the kit expiration date
	Washing Solution, Stopping Reagent	Close the vials tightly with screw caps and store them in the manufacturer's package.	Until the kit expiration date
	Calibrators 0-4, Control Serum, Conjugate, TMB-Substrate	Close the vials tightly with screw caps and store them in the manufacturer's package.	For two months

XV. GUARANTEE

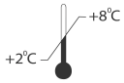
- Manufacturer guarantees conformity of the product to the requirements of regulatory and technical documentation.
- Quality and safety of the kit is guaranteed within established shelf life.
- Please contact Manufacturer if you have any questions.

AB Diagnostic Systems GmbH
Sportfliegerstraße 4, Berlin, 12487, Germany
Tel. +49 30 208987160, Fax: +49 30 208987199
E-Mail: info@ab-ds.de, www.ab-ds.de

XVI. REFERENCES

1. Lundberg P.R., Clin. Chem., Vol. 28, 1241 (1982).
2. Wild D., Immunoassay Handbook, Stockton Press, 339 (1994).
3. Ekins, "Free hormon assay". Nuclear Medicine Communications, 14, 676-688 (1993).

XVII. EXPLANATION OF SYMBOLS

	Manufacturer		
	Storage temperature limitation		Consult Instruction for use
	Date of manufacture CCYY-MM		For in vitro diagnostic use
	Expiry date CCYY-MM-DD		Sufficient for
	Batch code		Symbol "exclamation mark"
	Catalog number	**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 (blank)
2	**Add**	100 µl of Conjugate into all wells, except for the wells for OD control of TMB-Substrate (blank)
3	**Incubate**	60 min, microplate shaker (500-800 rpm), at room temperature
4	**Wash the plate**	**Working Washing Solution, 300 µl, 5 times**
5	**Add**	100 µl of TMB-Substrate into all wells
6	**Incubate**	20-30 min, at room temperature in a dark place
7	**Add**	150 µl of Stopping Reagent into all wells
8	**Mix**	5-10 seconds
9	**Read the optical density**	450 nm

pmol/l x 0.651 = pg/ml

pg/ml x 1.536 = pmol/l