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Hormone

AFIAS T4

INTENDED USE

AFIAS T4 is a fluorescence immunoassay (FIA) for the quantitative determination of total Thyroxine (total T4) in human serum/plasma. It is useful as an aid in management and monitoring of thyroid disorders.

For *in vitro* diagnostic use only.

INTRODUCTION

Thyroxine (T4) is one of two major hormones produced by the thyroid gland (the other is called triiodothyronine, or T3). T4 and T3 are regulated by a sensitive feedback system involving the hypothalamus and the pituitary gland. The hypothalamus releases the thyrotropin-releasing hormone (TRH), which stimulates the pituitary to release the thyroid stimulating hormone (TSH). This causes the thyroid to release T3 and T4 and these in turn regulate the release of TRH and TSH via a feedback control mechanism. Normally, elevated blood levels of T4 and T3 act to decrease the amount of TSH secreted, thereby reducing the production and release of T4 and T3. Over 99 % of T4 is reversibly bound to three plasma proteins in blood: thyroxine binding globulin (TBG) binds close to 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.

T4 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T4 decreases in hypothyroidism, myxedema and chronic thyroiditis (Hashimoto's disease). Increased levels of T4 have been found in hyperthyroidism due to Grave's disease and Plummer's disease.

PRINCIPLE

The test uses a competitive immunodetection method.

The antigen in the sample binds to the fluorescence-labeled detector antibodies in buffer, forming the complexes as a sample mixture.

They will migrate onto nitrocellulose matrix, which will interfere with the binding of the free fluorescence-labeled detector antibodies to the immobilized-antigen on a test strip.

More antigens in the sample will result in less free detection antibodies to accumulate, which lead to less fluorescence signal by the free fluorescence-labeled detector antibodies. This signal is processed by the instrument for AFIAS tests to show T4 concentration in the sample.

COMPONENTS

AFIAS T4 consists of 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each cartridge packaged in an aluminum pouch has three components including a cartridge part, a detector part and a diluent part.
- The cartridge part contains the membrane called a test strip which has T4-BSA conjugate at the test line, and streptavidin at the control line.
- The detector part has a granule containing anti-human T4-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).
- The diluent part contains ANS, tween 20, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect of test result(s).
- Do not reuse cartridges. A cartridge should be used for testing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if the pouch is damaged or have already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If cartridge and sample are stored in refrigerator, then allow cartridge and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for AFIAS tests may generate slight vibration during use.
- Used cartridges and pipette tips should be handled carefully and disposed by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **AFIAS T4** when biotin concentration in the sample was below 20 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **AFIAS T4** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS T4** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium Heparin

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Unopened
		1 month	Resealed

- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

MATERIALS SUPPLIED

REF SMFP-19

Components of **AFIAS T4**

- Cartridge box:
  - Cartridge 24
  - Pipette tip (zipper bag) 24
  - ID chip 1
  - Instructions for use 1
  - Spare cartridge zipper bag 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **AFIAS T4**. Please contact our sales division for more information.

- Instrument for AFIAS tests
  - **AFIAS-1** REF FRRR019
  - **AFIAS-3** REF FRRR040
  - **AFIAS-6** REF FRRR020
  - **AFIAS-10** REF FRRR038
- **Boditech Hormone Control** REF CFPO-95
- **Boditech Hormone Calibrator** REF CFPO-107
- **Boditech T4 Control** REF CFPO-237
- **Boditech T4 Calibrator** REF CFPO-263

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS T4** is human serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C or below.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the components of the **AFIAS T4** as described below. : Cartridges, pipette tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- If the sealed cartridge has been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for AFIAS tests.
- Empty the tip box.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for AFIAS tests operation manual for complete information and operating instructions.**

TEST PROCEDURE

▶ **AFIAS-1, AFIAS-3, AFIAS-6**

General mode

1) Insert the cartridge into the cartridge holder
2) Insert a tip into the tip hole of the cartridge.
3) Select the 'General mode' in the instrument for AFIAS tests.
4) Take 150 µL of the sample (serum/plasma/control) using a pipette and dispense it into the sample well of the cartridge.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 10 minutes.

▶ **AFIAS-10**

General mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Tap the 'Load' button of the bay that holds the cartridge with the tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
4) Insert the sample tube into the tube rack.
5) Insert the tube rack into the loading part of the sampling station.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 10 minutes.

Emergency mode – General tip

1) The test procedure is same with the 'Normal mode 1) – 3)'.
2) Convert the 'Emergency mode' in AFIAS-10.
3) Select the tip type (general tip) on the screen.
4) Select the sample type (serum/plasma) on the screen.
5) Take 150 µL of the sample using a pipette and dispense it into the sample well of the cartridge.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 10 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays T4 concentration of the test sample in terms of nmol/L.
- Working range: 10.23 - 300 nmol/L
- Conversion factor: 12.87 (SI: nmol/L = 12.87 x µg/dL)
- Reference range*: 57.9 - 150.6 nmol/L

* National institutes of health.

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **AFIAS T4**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical Sensitivity**
  - Limit of Blank (LoB) 7.18 nmol/L
  - Limit of Detection (LoD) 8.75 nmol/L
  - Limit of Quantitation (LoQ) 10.23 nmol/L
- **Hook effect**
  No high-dose effect is observed in this assay at T4 concentrations up to 1,800 nmol/L.
- **Analytical Specificity**
  - **Cross reactivity**
    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS T4** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactivity materials	Concentration
l-Triiodothyronine	500 ng/mL
reverse T3	500 ng/mL
l-Thyrosine	300 ng/mL
d-Thyrosine	300 ng/mL
3-Iodo-L-tyrosine	500 ng/mL
salicylic acid	1,000,000 ng/mL

  - **Interference**
    Interferents listed in the following table were added to the test sample(s) the same as the below concentrations listed below. **AFIAS T4** test results did not show any significant interference with these materials except for Cholesterol.
  - **AFIAS T4** does not recommend the use of lipid-rich samples.

Interference materials	Concentration
D-glucose	60 mM/L
L-Ascorbic acid	0.3 mM/L
Bilirubin(unconjugated)	0.7 mM/L
Hemoglobin	1000 mg/dL
Triglyceride	50 g/L
Biotin	20 ng/mL
Cholesterol	13 mM/L

- **Precision**
  - **Single site study**
    3 Lots of **AFIAS T4 we**re tested for 21 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.
    Repeatability (within-run precision)
    Within-laboratory precision (Total precision)
    Lot to lot precision

Expected value	Repeatability		Within-laboratory precision		Lot to lot precision	
[nmol/L]	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
50	50.19	6.9	50.34	6.6	49.89	6.3
100	101.48	7.2	100.94	6.8	100.42	6.6
200	197.72	6.6	199.07	6.6	200.14	6.5

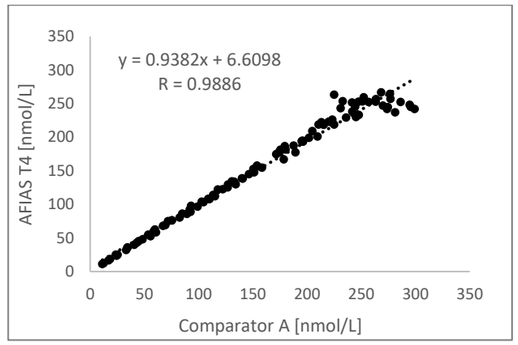
  - **Between persons**
    Three different persons tested three different lots of **AFIAS T4**, ten times at each concentration of the control standard.
  - **Between sites**
    One person tested **AFIAS T4** at three different sites, ten times at each concentration of the control standard.
  - **Between readers**
    One person tested **AFIAS T4** with three different readers, ten times at each concentration of the control standard.

Expected Value	Between person		Between site		Between reader	
[nmol/L]	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
50	49.51	6.2	50.55	5.8	49.36	6.5
100	99.46	5.2	98.29	5.7	99.13	5.0
200	199.91	6.7	201.14	6.2	197.94	6.5

- **Accuracy**
  The accuracy was confirmed by testing with 3 different lots of **AFIAS T4**. The tests were repeated 10 times at each concentration of the control standard.

Expected value [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
200.00	197.94	196.97	196.16	197.02	98.5
170.00	171.66	170.07	172.97	171.57	100.9
140.00	139.94	137.51	144.32	140.59	100.4
110.00	110.24	110.99	112.72	111.32	101.2
80.00	78.58	81.07	81.64	80.43	100.5
50.00	48.60	49.26	50.75	49.54	99.1

- **Comparability**
  T4 concentration of 100 clinical samples were quantified independently with **AFIAS T4 (AFIAS-6)** and **comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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Note: Please refer to the table below to identify various symbols.

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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