Hormone

AFIAS Free T4

INTENDED USE

AFIAS Free T4 is a fluorescence immunoassay (FIA) for the quantitative determination of Free T4 (Free thyroxine) in human whole blood/serum/plasma. It is useful as an aid measurement the assessment of thyroid function.

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.

Free thyroxine acts directly on peripheral tissues as deiodinase within cells to form T3 and binding to the nucleus to indicate the action of hormones. Unlike total thyroxine, it is not affected by TBG, and with some exceptions, if there is an abnormality in free thyroxine, it can be considered as thyroid dysfunction, so the thyroid function.

PRINCIPLE

The test uses a competitive immunodetection method.

The antigen in the sample bind 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 the 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 Free T4 concentration in the sample.

COMPONENTS

AFIAS Free 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 streptavidin at the test line, and chicken IgY at the control line.
- The detector part has 2 granules containing anti-T4-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, T3-biotin conjugate and sodium azide as a preservative in Tris-HCl.
- The diluent part contains sodium azide as a preservative in Tris-HCl.

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 a cartridge, if the pouch is damaged or has 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 test components and/or 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, C-tips 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 it may cause certain health issues like convulsions, low blood pressure, low 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 Free T4** when biotin concentration in the sample was below 5 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 Free T4** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS Free T4** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Sodium Heparin

- **C-tip should be used when the following conditions are met.**
  - C-tip provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Do not perform a test with C-tip on General Mode. It might cause an erroneous result.
  - Excess whole blood around the C-tip should be wiped off.
  - In order to avoid cross-contamination, please do not re-use C-tip for multiple samples.
  - AFIAS cartridge should be inserted and positioned in the cartridge holder prior to the blood sample collection.
  - While collecting blood, be careful not to create air bubbles in the C-tip.

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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes the antigens 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-145

Components of **AFIAS Free 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 with **AFIAS Free T4**.

Please contact our sales division for more information.

- Instrument for AFIAS tests
  - **AFIAS-1** REF FPRR019
  - **AFIAS-3** REF FPRR040
  - **AFIAS-6** REF FPRR020
  - **AFIAS-10** REF FPRR038
- **C-tip (30 µL)** REF CFPO-199
- **Boditech Free T4 Control** REF CFPO-375
- **Boditech Free T4 Calibrator** REF CFPO-376

SAMPLE COLLECTION AND PROCESSING

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

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 24 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for 1 week at 2-8°C prior to being tested. If testing will be delayed more than 1 week, samples should be frozen at -20°C.
- The samples (serum, plasma) stored frozen at -20°C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Collection of whole blood sample using C-tip.
  1. Hold the C-tip horizontally and touch the surface of the blood with the tip of the C-tip.
  2. Capillary action will automatically draw the blood sample to C-tip and stop.
  3. Wipe off any excess blood around the tip.
  4. Double-check if whole blood is filled accurately in the C-tip and AFIAS reader is ready for a test on the 'C-tip mode'.

TEST SETUP

- Check the components of the **AFIAS Free T4** as described below. : Cartridges, pipette tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the ID chip.
- 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 200 µL of sample (whole blood/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 12 minutes.

C-tip mode
1) Insert the cartridge into the cartridge holder.
2) Take 30 µL of whole blood using a C-tip.
3) Insert the whole blood-filled C-tip into the tip hole of the cartridge.
4) Select the 'C-tip mode' in the instrument for AFIAS tests.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 12 minutes.

▶ **AFIAS-10**

Normal 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 12 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 (whole blood/serum/plasma) on the screen.
5) Take 200 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 12 minutes.

Emergency mode – C-tip
1) Insert a cartridge into the cartridge holder.
2) Take 30 µL of whole blood using a C-tip.
3) Insert the C-tip with sample into the tip hole of the cartridge.
4) Tap the 'Load' button of the bay that holds the cartridge with a tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
5) Convert the 'Emergency mode' in AFIAS-10.
6) Select the tip type (C-tip) on the screen.
7) Tap the 'Start' button on the screen.
8) The test result will be displayed on the screen after 12 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays Free T4 concentration of the test sample in terms of pmol/L.
- Working range: 1 – 100 pmol/L
- Conversion factor: 12.87 (SI: pmol/L = 12.87 x ng/dL)
- Reference range: 9 – 22 pmol/L

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 Free 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) 0.54 pmol/L
  - Limit of Detection (LoD) 0.86 pmol/L
  - Limit of Quantitation (LoQ) 1.00 pmol/L

- **Analytical Specificity**
  - **Cross reactivity**

Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS Free 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-Tyrosine	300 ng/mL
D-Tyrosine	300 ng/mL
3-iodo-L-tyrosine	500 ng/mL

- **Interference**

Interferents listed in the following table were added to the test sample(s) the same at the concentration mentioned below. **AFIAS Free T4** test results did not show any significant interference with these materials except for cholesterol.

AFIAS Free T4 does not recommend the use of lipid-rich samples.

Interferents	Concentration
D-glucose	60 mM/L
L-Ascorbic acid	0.3 mM/L
Bilirubin(unconjugated)	0.7 mM/L
Hemoglobin	1,000 mg/dL
Triglyceride	50 g/L
Cholesterol	13 mM/L
salicylic acid	1,000 µg/mL

- **Precision**
  - **Single-site study**

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **AFIAS Free T4** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study					
Repeatability		Within-laboratory precision		Lot to lot precision	
Mean [pmol/L]	CV (%)	Mean [pmol/L]	CV (%)	Mean [pmol/L]	CV (%)
6.43	12.84	6.41	12.90	6.35	13.86
12.83	14.76	12.63	14.98	12.51	15.15
48.54	8.89	49.96	8.69	50.11	8.31

  - **Multi-site study**

Reproducibility

1 Lot of **AFIAS Free T4** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study	
Reproducibility	
Mean [pmol/L]	CV (%)
6.20	14.86
12.35	14.64
49.23	9.61

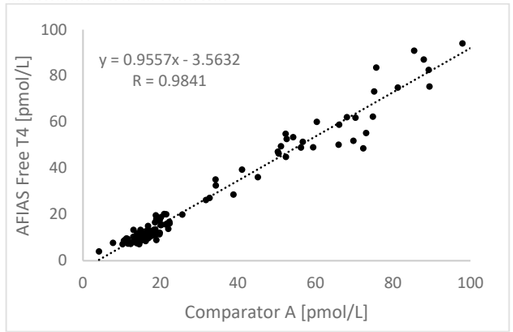
- **Accuracy**

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

Expected value [pmol/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
50.00	49.62	49.50	52.30	50.48	101.0
41.25	40.36	41.60	41.47	41.14	99.7
32.50	33.60	34.11	32.65	33.45	102.9
23.75	23.01	24.79	24.86	24.22	102.0
15.00	14.35	15.58	16.79	15.57	103.8
6.25	5.68	6.88	6.46	6.34	101.4

- **Comparability**

Free T4 concentration of 100 clinical samples were quantified independently with **AFIAS Free 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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