IVD CE

FREE THYROXINE (F-T4) ELISA KIT

Catalog No. E1005

INTENDED USE

The Autobio f-T4 assay is designed for the quantitative determination of free thyroxine (f-T4) concentration in human serum.

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.[1,2] Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy.[3] Under normal thyroid condition, as the concentrations of the carrier proteins alter, the total thyroxine level changes so that the free thyroxine concentration remains constant.[4] Thus, measurement of free thyroxine concentrations correlate better with clinical status than total thyroxine levels.

For example, the increase in total thyroxine associated with pregnancy, oral contraceptives and estrogen therapy occasionally result in total T4 levels over the limits of normal while the free thyroxine concentation remains within the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration. The total T4 can be elevated or lowered by TBG changes such that the normal reference levels result is observed. Again, the free thyroxine concentration typically uncovers the patient's actual clinical status.

PRINCIPLE OF THE TEST

In the f-T4 EIA, a certain amount of T4 analog is coated on microtiter wells. A measured amount of patient serum, and a constant amount of anti-T4 antibody conjugated with horseradish peroxidase are added to the microtiter wells. During the incubation, T4 analog on microtiter wells and f-T4 present in the samples and reference standards compete for binding to the anti-T4 monoclonal antibody-horseradish peroxidase conjugate. After 60 minutes incubation at 37 ℃, the wells are washed by wash solution. Then substrate solution and chromogen solution are added and incubated for 20 minutes, resulting in the development of a blue color. The color development is terminated with the addition of stop solution, and the color is changed to yellow and absorbance is measured spectrophotometrically at 450 nm. The color intensity is inversely related to the concentration of f-T4 in the test sample.

MATERIALS PROVIDED

1. FT4 Coated Microwell: Microplate with T4 analog coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-T4 antibody in stabilizing buffer (1 vial, 11 ml)
3. Reference Standards: 0, 5.0, 10, 25, 50, 100 pmol/l f-T4 in human plasma with preservatives. (6 vials, 1 ml/ea)
4. Wash Solution Concentrate: PBS-Tween (1 bottle, 25 ml, 40X)
5. Substrate Solution: Hydrogen peroxide (1 vial, 7.5 ml)
6. Chromogen Solution: Tetramethylbenzidine (TMB) (1 vial, 7.5 ml)
7. Stop Solution: 1.0 M H_2SO_4 (1 vial, 7.5 ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit:

1. Distilled water
2. Precision pipettes for delivery of 20-200 µl, 100-1000 µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Microplate ELISA reader with a bandwidth of 10nm or less and an absorbance range of 0-3.5 or greater at 450 nm wavelength
4. Magnetic stirrer
5. Washer for microplates
6. Quality control specimens
7. Incubator
8. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2～8 ℃ upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable for at least two months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARETION

1. Serum is the recommended sample type for this assay. Samples collected in tubes with EDTA and Sodium Citrate as an anticoagulant may interfere with test procedures as they inhibit the enzyme reaction and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2～8 ℃. For longer storage, freeze the specimens at -20 ℃. Thawed samples must be mixed prior to testing and multiple freeze-thaw cycles must be avoided.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents, Stop Solution contains H_2SO_4, in case of contact, wash thoroughly with water.
5. Sodium azide in reference standards can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.
6. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18～25 ℃) before use.
2. Adjust the incubator to 37 ℃.
3. Prepare Wash Solution: add 25 ml of Wash Solution Concentrate to 1000 ml of distilled water, and mix well with a magnetic stirrer. The Wash Solution is stable at room temperature for two months.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all Reference Standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50 μl of standards, samples, and controls into appropriate wells.
3. Dispense 100 μl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
5. Incubate at 37 ℃ for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter plate 5 times with wash solution.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 50 μl of Chromogen Solution into each well.

10. Dispense 50 μl of Substrate Solution into each well. Gently mix for 15 seconds.
11. Incubate at room temperature in the dark for 20 minutes without shaking.
12. Stop the reaction by adding 50 μl of Stop Solution to each well.
13. Gently mix for 15 seconds. It is very important to make sure that the blue color changes to yellow color completely.
14. Read the absorbance at 450 nm with a microplate ELISA reader within 15 minutes.

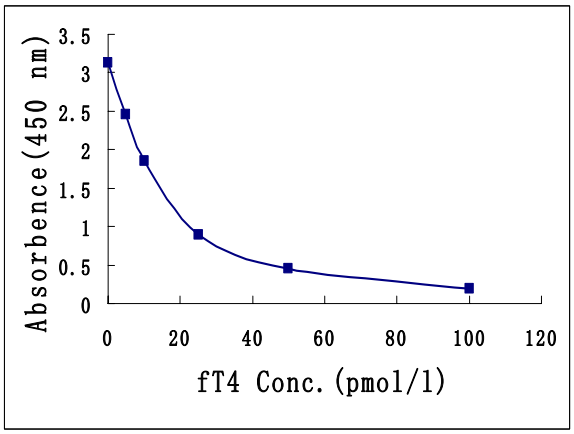
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the absorbance values (ordinate) for each Reference Standard against the corresponding concentration of f-T4 in pmol/l (abscissa) and draw a calibration curve through the Reference Standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

EXAMPLE OF CALIBRATION CURVE

A typical calibration curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

f-T4 (pmol/l)	Absorbance (450 nm)
0	3.135
5.0	2.455
10	1.859
25	0.899
50	0.454
100	0.189



EXPECTED VALUES

Each laboratory should establish its own normal range. These values are given only for guidance.

Sample Number	120
Average Value（pmol/l）	16.02
Standard Deviation (σ)	3.53
Normal Range (±2σ, pmol/l)	9.0-23.0

PERFORMANCE

A. Sensitivity

Twenty zero standards were assayed along with a set of other Reference Standards. The detection limit, defined as the apparent concentration corresponding to two standard deviations below the average absorbance at zero binding, was determined to be not higher than 2.5 pmol/l.

B. Specificity

The cross-reactivity of the free T4 assay kit with T3 and r-T3 was determined by adding these hormones to zero standards. The color intensity produced was then determined.

Interferent	Concentration	Measured Value (pmol/l)	Crosstalk Rate (%)
T3	1000 ng/ml	10.56	0.00082
r-T3	300 ng/ml	11.44	0.00296

C. Precision

a. Intra-assay Precision

Intra-assay precision was determined by assaying 20 replicates of each of 2 control sera; low and high.

Serum	Number	Mean	SD	RSD（%）
Low	20	8.61	0.59	6.87
High	20	20.20	0.84	4.14

b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of each of 2 serum pools in 20 separate runs, using a calibration curve constructed for each run.

Serum	Number	Mean	SD	RSD（%）
Low	20	8.74	0.63	7.25
High	20	20.23	1.30	6.47

D. Accuracy

For 175 samples in the range of 3.0 pmol/l to 82.0 pmol/l, the relationship between the Autobio f-T4 ELISA test and the Bayer ADVIA Centaur® f-T4 CLIA test is described by the equation below:

Reference	No. of Specimens	Least Square Regression Analysis	Correlation Coefficient
Bayer ADVIA Centaur®（CLIA）	175	y =1.0575x－1.5094	0.932

LIMITATIONS

1. As with all immunoassays, the results of this test can be influenced by factors present in some patients' specimens. The reagents for this assay have been formulated to minimise interference from heterophilic antibodies and from nonspecific protein binding. However, individual sample results may be affected.
2. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.
3. Procedural directions must be followed exactly as any modification of the procedure may change the results.
4. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop human anti-mouse antibodies (HAMA). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies.[5,6] Samples containing HAMA should not be assayed with the free T4 assay.
5. If a patient, for some reason, reads higher than the highest reference standard report as such (e.g. ＞100 pmol/l). **Do not try to dilute the sample. TBG variations in different matrices will not allow free T4 hormone to dilute serially.**
6. Serum free-thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG. Thus, free-thyroxine concentration alone is not sufficient to assess the clinical status.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

LOT	**BATCH CODE**
	USE BY
	MANUFACTURER
EC REP	**AUTHORISED REPRESENTATIVE WITHIN THE EUROPEAN COMMUNITY**
Σ	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
2 °C 8 °C	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	CONSULT INSTRUCTIONS FOR USE

REFERENCES:

1. Ingbar, S.H., Woeber, K.A., The Thyroid Gland, in: Textbook of of: Endocrinology, 6th ed., Williams, R.H., (ed.) W.B. Saunders Co. Philadelphia, pp 117-247, 1981.
2. Ray, R.A., Howanitz, P., RIA in Thyroid Function Testing, Diag. Med., May 1984 pp. 55-70.
3. Larsen, P.R., Triiodothyronine: Review of Recent Studies of its Physiology and Pathophysiology in Man, Metabolism 21: 1073, 1972.
4. Schussler, G.C., Nonthyroidal Illness in the Thyroid, 5th ed. S.H. and Braverman L.E. (eds.), J.B. Lippincott Co., Philadelphia, pp 381-406.
5. KINDERS, R.J., HASS, G.M., Interference in immunoassay by human anti-mouse antibodies. Eur. J. Cancer. 26 (5):647, 1990.
6. BOSCATO, L.M., STUART, M.C., Incidence and specificity of interference in two-site immunoassays. Clin. Chem. 32 (8): 1491, 1986.

For orders and inquires, please contact

EC REP	Wellkang Ltd. ADD: Suite B, 29 Harley Street, LONDON, W1G 9QR, U.K. Tel:+44(20) 79934346, 88168300 Fax:+44(20)76811874 Web: www.CE-marking.com www.CE-marking.org www.CE-marking.eu
	AUTOBIO DIAGNOSTICS CO., LTD. ADD: No.87 Jingbei Yi Road, National Eco & Tech Development Area, Zhengzhou , China 450016 Tel: +86-371-67985313 Fax: +86-371-67985804 Web: www.autobio.com.cn