IVD CE

FREE TRIIODOTHYRONINE (F-T3) ELISA KIT

Catalog No. E1004

INTENDED USE

The Autobio free triiodothyronine (f-T3) ELISA test kit is intended for the quantitative determination of free triiodothyronine (f-T3) concentration in human serum.

INTRODUCTION

Triiodothyronine (T3), a thyroid hormone, circulates in blood almost completely bound (>99.5%) to carrier proteins. The main transnport protein is thyroxine-binding globulin (TBG)[1, 2]. However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Further, 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 alter, the total triiodothyronine level changes so that the free triiodothyronine concentrations remains constant. Thus, measurements of free triiodothyronine concentrations correlate more reliably with clinical status than total triiodthyronine levels. For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T3 levels while the free T3 concentration remains basically unchanged[3, 4].

This ELISA test provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of free T3.

PRINCIPLE OF THE TEST

In the freeT3 EIA, a certain amount of T3 analog is coated on microtiter wells. A measured amount of patient serum, and a constant amount of anti-T3 antibody conjugated with horseradish peroxidase are added to the microtiter wells. During the incubation T3 analog on microtiter wells and free T3 present in the samples and reference standards compete for binding to the anti-T3 monoclonal antibody-horseradish peroxidase conjugate. After a 60 minute incubation at 37℃, the wells are washed by wash solution. Substrate solution and chromogen solution are then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, then the color is changed to yellow and the absorbance is measured spectrophotometrically at 450 nm. The color intensity is inversely related to the concentration of free T3 in the test sample.

MATERIALS PROVIDED

1. FT3 Coated Microwell: Microplate with T3 analog coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled antibodies to T3 (anti-T3) in Stabilizing Buffer (1 vial, 11ml)
3. Reference Standards: 0, 2, 5, 10, 25, 50pmol/l free T3 in human plasma with preservatives. (6 vials, 1ml/ea)
4. Wash Solution Concentrate: PBS-Tween (1 bottle, 25ml, 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 20-200µl, 100-1000µl (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 450nm 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 PREPARATION

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures 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. Multiple freeze-thaw cycles should 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. The Reference Standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the Reference Standards and 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 copious amount 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 brought to room temperature (18～25℃) before use.
2. Adjust the incubator to 37℃.
3. Prepare Wash Solution: add 25ml of Wash Solution Concentrate to 1000ml 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 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. Prepare data sheet with sample identification.
2. Dispense 50μl of Reference Standards, samples, and controls into appropriate wells.
3. Dispense 100μl 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 wells 5 times with Wash Solution.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 50μl Chromogen Solution into each well.
10. Dispense 50μl 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 50μl 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 completely.
14. Read absorbance at 450nm with a Microplate ELISA reader within 15 minutes.

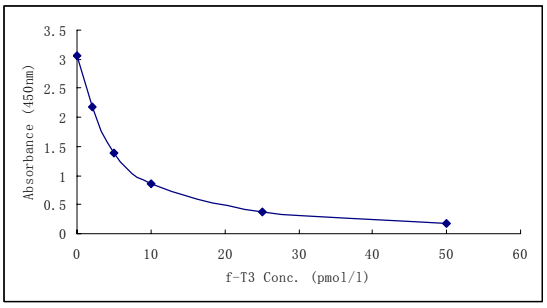
CALCULATION OF RESULTS

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 free T3 in pmol/l (abscissa) and draw a standard curve by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the standard 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.

Free T3 (pmol/l)	Absorbance (450nm)
0	3.05
2	2.18
5	1.49
10	0.82
25	0.37
50	0.24



EXPECTED VALUES

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

Sample Numbers	156
Average Value（pmol/l）	5.01
Standard Deviation (σ)	0.76
Normal Range (±2σ, pmol/l)	3.5-6.5

PERFORMANCE

A. Sensitivity

Twenty zero Reference 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 not higher than 0.8pmol/l.

B. Specificity

The cross-reactivity of the free T3 assay kit with T4 and rT3 was determined by adding these hormones to zero standards. The color intensity produced was then determined.

Interferent	Concentration	Measured Value (pmol/l）	Crosstalk Rate (%)
T4	500ng/ml	1.32	<0.001
rT3	500ng/ml	1.48	<0.001

C. Precision

a. Intra-assay Precision

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

Serum	Number	Mean	SD	RSD（%）
Low	20	4.54	0.27	5.92
High	20	13.59	0.96	7.08

b. Inter-assay Precision

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

Serum	Number	Mean	SD	RSD（%）
Low	20	4.87	0.41	8.32
High	20	13.21	0.61	4.61

D. Accuracy

For 70 samples in the range of 1.5pmol/l to 45pmol/l, the relationship between the Autobio freeT3 ELISA Test and the Bayer ADVIA Centaur® Free T3 (CLIA) Test is described by the equation below:

Reference	Number of Specimens	Least SquareRegression Analysis	Correlation Coefficient
Bayer ADVIA Centaur®	70	Y=0.9568x + 0.5091	0.979

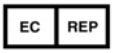
LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. If a patient, for some reason, reads higher than the highest reference standard report as such (e.g. > 50 pmol/l). **Do not try to dilute the sample. TBG variations in different matrices will not allow Free T3 hormone to dilute serially.**
4. Several drugs are known to affect the binding of triiodothyronine to the thyroid hormone carrier proteins or its metabolism to T3 and complicate the interpretation of free T3 results.
5. Circulating autoantibodies to T3 and hormone-binding inhibitors may interfere.
6. 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.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each standard 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.

SYMBOLS

	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**AUTHORISED REPRESENTATIVE WITHIN THE EUROPEAN COMMUNITY**
	**CONTAINS SUFFICIENT FOR <n> TESTS**

IVD	**IN VITRO DIAGNOSTIC MEDICAL DEVICE**
8 °C 2 °C	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	CONSULT INSTRUCTIONS FOR USE

REFERENCES:

1. Barker, S.B. "Determination of Protein Bound Iodine." Journal Biological Chemistry, 173, 175(1948).
2. Chopra, I.J, Solomon, D.H and Ho, R. S. "A Radioimmunoassay of Thyroxine", J. Clinical Endocrinol. 33, 865 (1971).
3. Young, D.S, Pestaner, L.C, and Gilberman, U. "Effects of Drugs on Clinical Laboratory Tests", Clinical Chemistry, 21, 3660(1975)
4. Sterling, L. Diagnosis and Treatment of Thyroid Disease, Cleveland, CRC Press, P. 19-51(1975)

For order and inquires, please contact

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