IVD

FREE TRIIODOTHYRONINE (F-T3) CHEMILUMINESCENCE

IMMUNOASSAY KIT

Catalog No. CL1004-2

INTENDED USE

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

INTRODUCTION

Triiodothyronine, 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]. Howerer, 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 CLIA test provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of free T3.

PRINCIPLE OF THE TEST

In this free T3 assay, 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 sample and standard compete for binding to the anti-T3 monoclonal antibody-horseradish peroxidase conjugate. After a 60 minute incubation at 37°C, the wells are washed 5 times by wash buffer to remove unbound anti-T3-antibody conjugate. The concentration of free T3 in a patient sample or control is then determined by interpolation from the standard curve. The Related Light Unit (RLU) is directly proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fT3 in the sample. By reference to a series of fT3 standards assayed in the same way, the concentration of fT3 in the unknown sample is quantified.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: Microplate with T3 analog coated wells (1 plate, 48wells/96 wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled anti-T3 in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 0, 2, 5, 10, 25, 50 pmol/l free T3 in human plasma with preservatives. (6 vials, 0.5ml/ea)
4. PBS-T powder: PBS-Tween (1 bag, 5g)
5. Substrate A: (1 vial, 3.5ml/6.0ml)
6. Substrate B: (1 vial, 3.5ml/6.0ml)

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. Luminometer
4. Magnetic stirrer
5. Vortex Mixer or equivalent
6. Washer for microplates
7. Quality control specimens

8. Incubator
9. 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.

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.
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 brought to room temperature (18-25℃) before use.
2. Adjust the incubator to 37℃.
3. Prepare Wash Solution: add 1 bag of PBS-T powder to 500ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for 2 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 invalid results.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Dispense 50μl of 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 plate 5 times with wash solution
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.

9. Dispense 50μl of Substrate A, then 50μl of Substrate B into each well. Gently mix for 10 seconds.
10. Put the microtiter plate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well

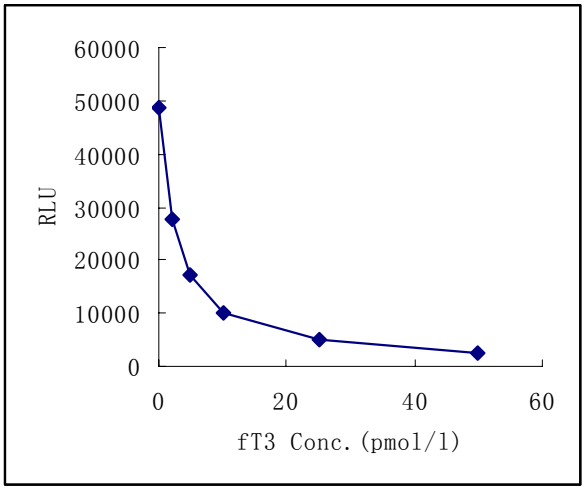
CALCULATION OF RESULTS

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 RLU (ordinate) for each reference standard against the corresponding concentration of fT3 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 STANDARD CURVE

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

fT3 (pmol/l)	RLU
0	48728
2	27694
5	17117
10	10272
25	4936
50	2487



EXPECTED VALUES

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

Sample Numbers	131
Average Value（pmol/l）	5.1
Standard Deviation (σ)	0.76
Normal Range (±2σ, pmol/l)	3.5~6.5

PERFORMANCE

A. Sensitivity

Twenty zero standards were assayed along with a set of other standards. The sensitivity, defined as the apparent concentration corresponding to two standard deviations below the average RLU at zero binding, was lower than 0.8pmol/l.

B. Specificity

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

Interferent	Concentration	Measured Value(pmol/l)	Crosstalk Rate (%)
T4	500ng/ml	1.09	<0.001
rT3	500ng/ml	1.56	<0.001

C. Precision

a. Intra-Assay Precision

Intra-Assay Precision was determined by assaying 20 replicates of each of 2 sera; low and high.

Serum	Number	Mean	SD	RSD（%）
Low	20	4.68	0.30	6.34
High	20	13.87	0.84	6.06

b. Inter-Assay Precision

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

Serum	Number	Mean	SD	RSD（%）
Low	20	4.65	0.33	7.17
High	20	13.85	0.96	6.96

D. Accuracy

For 245 samples in the range of 1.5pmol/l to 45pmol/l, the relationship between the Autobio freeT3 CLIA Test and the Bayer ADVIA Centaur® Free T3 (CLIA) Test is described by the equation:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Bayer	245	Y=1.0406X + 0.7705	0.972

LIMITATIONS

This assay has not been validated for newborn testing.

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.

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.

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 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

	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**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)
5) Boscato LM， Stuart MC.Heterophilic antibodies: a problem for all immunoassays.Clin Chem 34:27-33(1988)

For order , please contact

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