IVD

FREE THYROXINE (f-T4) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL1005-2

INTENDED USE

The Autobio f-T4 CLIA test kit is intended 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)[2]. However, only the free (unbound) portion of thyroxine is responsible for the biological action[3]. Further more, 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 thyroxine level changes so that the free thyroxine concentration remains constant[1,4]. 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 in the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration[5]. The total T4 can be elevated or lowered by TBG changes such that the test result would not be able reflect the actual thyroid status. Again, the free thyroxine concentration typically uncovers the patient's actual clinical status.

PRINCIPLE OF THE TEST

In the fT4 CLIA test kit, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the f-T4 and f-T4 enzyme conjugate compete for the limited binding sites on the anti-T4 antibody. After 60 minutes incubation at 37℃, the wells are washed by Wash Solution to remove unbound f-T4 enzyme conjugate. The Related Light Unit (RLU) is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled f-T4 in the sample. By reference to a series of f-T4 standards assayed in the same way, the concentration of f-T4 in the unknown sample is quantified.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate with anti-T4 Antibody coated wells (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled T4 in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 0, 0.2, 0.5,1.5, 4.5, 9ng/dl f-T4 in HEPES solution with preservatives. (6 vials, lyophilized)
4. Substrate A: (1 vial, 3.5ml/6.0ml)
5. Substrate B: (1 vial, 3.5ml/6.0ml)
6. PBS-T Powder: PBS-Tween (1 bag, 5g)

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. Vortex Mixer or equivalent
5. Washer for microplate
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. 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. Reconstituted standards should be used within 30 days and be frozen at -20℃ for long term storage. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 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 canbe 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. 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.
5. 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℃) prior to use.
2. Adjust the incubator to 37℃
3. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2～8℃.
4. Prepare Wash Solution: Add 1 bag of PBS-T Powder to 500 ml of distilled water, and mix well. 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. Make 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 micro 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 microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

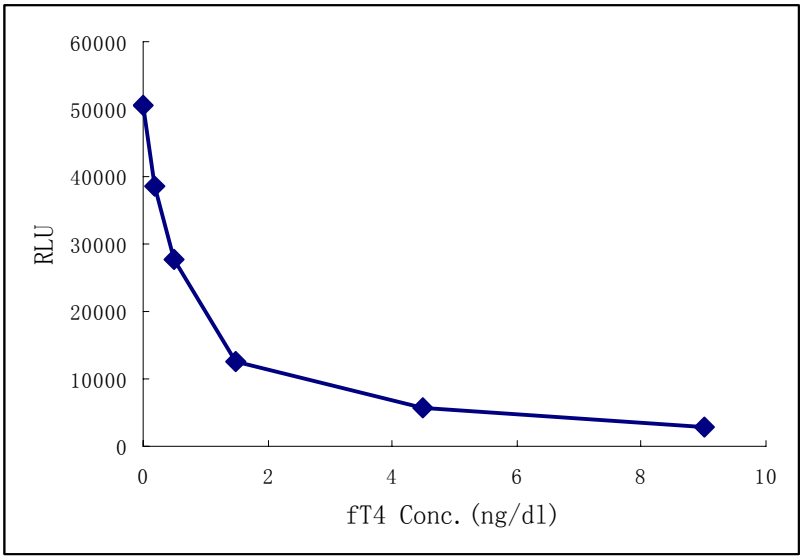
CALCULATION OF RESULTS

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 f-T4 in ng/dl (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.

f-T4 (ng/dl)	RLU
0	50566.5
0.2	38603.5
0.5	27665.2
1.5	12650.5
4.5	5771.2
9	2797.36



EXPECTED VALUES

A normal range of 0.88 ng/dL to 1.85ng/dL (central 95% interval) was obtained by testing serum specimens from 287 individuals determined as normal by Autobio TSH CLIA kit and Autobio free T4（f-T4）CLIA kit. It is recommended that each laboratory establishes its own normal range.

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 0.07ng/dl.

B. Specificity

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

Interferent	Concentration	Measured Value (ng/dl)	Crosstalk Rate (%)
T3	500ng/ml	0.19	<0.01%
rT3	500ng/ml	0.27	<0.01%

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	0.92	0.05	5.84
High	20	3.37	0.18	5.46

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	0.89	0.06	6.78
High	20	3.41	0.24	6.93

D. Accuracy

For 193 samples in the range of 0.22μg/dl to 8.76ng/dl, the relationship between the Autobio free T4(f-T4) CLIA Test and the Siemens ADVIA Centuar free T4 Test is described by the equation below:

Reference	No. of Specimens	Least Square Regression Analysis	Correlation Coefficient
Siemens ADVIA Centuar free T4	193	Y=0.973x + 1.3725	0.938

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. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU reading.

Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with in vitro immunoassays[6]. 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 form this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

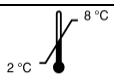
Serum free-thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A decrease in free thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect free thyroxine values, has been compiled by the *Journal of the American Association of Clinical Chemists*.

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**
	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

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3. Surk M L, Chopra I J, Mariash C N. et a1. American thyroid association guidelines for use of laboratory tests in thyroid disorders [J]. JAMA, 1990, 263: 1529-1532.
4. Wilke, T.J. 1986. Estimation of free thyroid hormone concentrations in the clinical laboratory. Clinical Chemistry, 32(4): 585-592.
5. Demem L M, Spencer C A. Laboratory medicine practice guidelines for laboratory support for the diagnosis and monitoring of thyroid diseases [J]. National Academy of Clinical Biochemistry, 2002.
6. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27-33.

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