Annex 1

Instruction for Use

TRIIODOTHYRONINE (T3) ELISA KIT

Catalog No. E1001

INTENDED USE

The Autobio T3 assay is designed for the quantitative determination of Triiodothyronine (T3) concentration in human serum.

INTRODUCTION

The Human thyroid gland is a major component of the endocrine system. Thyroid hormones perform many important functions. They exert powerful and essential regulatory influences on growth, differentiation, cellular metabolism and general hormonal balance of the body, as well as on the maintenance of metabolic activities and the development of the skeletal and organ system.[1]

The hormones thyroxine (T4) and 3, 5, 3' thiiodothyronine (T3) circulate in the bloodstream, mostly bound to the plasma protein, thyroxine binding globulin (TBG).[2,3] The concentration of T3 is much less than that of T4, but its metabolic potency is much greater.

T3 determination is an important factor in the diagnosis of thyroid diseases.[4] Its measurement has uncovered a variant of hyperthyroidism in thyrotoxic patients with elevated T3 levels and normal T4 levels. An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. The clinical significance of T3 is also evident in patients in whom euthyroidism is attributable only to normal T3, although their T4 values are subnormal.

T3 determination is also useful in monitoring both patients under treatment for hyperthroidism and patients who have discontinued anti-thyroid drug therapy. It is especially valuable in distinguishing between euthyroid and hyperthyroid subjects.

In addition to hyperthyroidism, T3 levels are elevated in women who are pregnant, and in women receiving oral contraceptives or estrogen treatment, paralleling TBG increases in a manner analogous to T4 levels. Likewise, a reduction in TBG concentration decreases T3 concentration. These changes in the T3 level, however, are not a true reflection of thyroid status.

PRINCIPLE OF THE TEST

In the T3 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. 8-anilino-1-napthalene sulfonate (ANS) is used to displace T3 from proteins to enable the measurement of total circulating T3. During the incubation T3 analog on microtiter wells and T3 present in the samples and 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 is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of Stop Solution, and 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 T3 in the test sample.

MATERIALS PROVIDED

1. Antigen Coated Microtiter Plate: Microplate with T3 analog coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Concentrate: Horseradish Peroxidase (HRP) labeled anti-T3 in Stabilizing Buffer (1 vial, 1.2 ml, 20X)
3. Enzyme Conjugate Diluent (2 vials, 7.5 ml/ea)
4. Reference Standards: 0, 0.5, 1.0, 2.5, 5.0, 10ng/ml T3 in human plasma with preservatives. 1ng/ml of the reference standard is equivalent to 1ng/ml standard of the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), P.R.C. (6 vials, 1ml/ea)
5. Wash Solution Concentrate: PBS-Tween (1 bottle, 25ml, 40X)
6. Substrate Solution: Hydrogen Peroxide (1 vial, 7.5 ml)
7. Chromogen Solution: Tetramethylbenzidine (1 vial, 7.5 ml)
8. Stop Solution: 1.0M 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 optical density range of 0-3.5 OD or greater at 450nm wavelength
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 microplate 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 (One 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 calibrators 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 calibrators 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 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 Enzyme Conjugate Reagent: add 0.1 ml of Enzyme Conjugate Concentrate to 2.0 ml of Enzyme Conjugate Diluents (1:20 dilution), and mix well. The amount of conjugate diluted is depending on your assay size. The Conjugate Reagent is stable at 4℃ for 7 days.
4. Prepare Wash Solution: add 25ml of Wash Solution Concentrate to 1000ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at R.T. 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.
6. When the Enzyme Conjugate Concetrate is diluted in the Enzyme Conjugate Diluent, the obtained Conjugate Reagent is stable at 4℃ for 7 days. We recommend the amount of Enzyme Conjugate Concentrate diluted is depending on your assay size.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Prepare 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 wells 5 times with wash solution.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 50 μl Chromogen Solution into each well.
10. Dispense 50 μl 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.

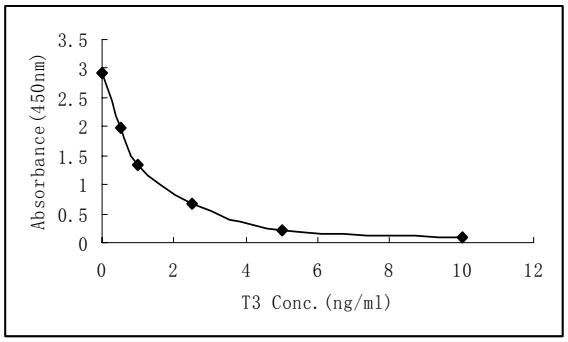
CALCULATION OF RESULTS

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 calibrator against the corresponding concentration of T3 in ng/ml (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolation 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.

T3 (ng/ml)	Absorbance (450nm)
0	2.91
0.5	1.972
1.0	1.347
2.5	0.671
5.0	0.225
10.0	0.085



EXPECTED VALUES

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

Sample Numbers	105
Average Value（ng/ml）	1.34
Standard Deviation (σ)	0.27
Normal Range (±2σ, ng/ml)	0.80-1.90

PERFORMANCE

A. Sensitivity

Twenty zero standards were assayed along with a set to other 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 0.2ng/ml.

B. Specificity

The cross-reactivity of the total 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 (ng/ml）	Crosstalk Rate (%)
T4	500ng/ml	0.64	0.128
rT3	500ng/ml	0.32	0.064

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	0.94	0.037	3.94
High	20	6.69	0.377	5.63

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	0.91	0.071	7.83
High	20	6.76	0.677	10.01

D. Accuracy

For 95 samples in the range of 0.4ng/ml to 8.5ng/ml, the relationship between the Autobio T3 ELISA Test and the Biocheck T3 ELISA Test is described by the equation:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Biocheck	95	Y=0.9295X – 0.2858	0.963

（ELISA）			

LIMITATIONS

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.

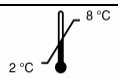
Serum T3 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of T3 to TBG. Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.

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

REFERENCES:

[1]Chopra IJ, Ho RS, Lam R. An improved radio-immunoassay of triodothyronine in hunan serum. J Lab Clinical Med, 80, 729 (1971)

[2]Young DS, Pestaner LC, and Gilberman. Effects of Drugs on Clinical Laboratory Test. Clinical Chemistry. 21, 3660 (1975)

[3]Sterling L, Diagnosis and treatment of Thyroid Disease, Cleveland, CRC Press, 9, 51 (1975)

[4]Schroeder HR**,** Boguslaski RC, Carrico RJ, Buckler RT. Monitoring specific protein-binding reactions with chemiluminescence. Methods Enzymol 1978; 57: 424-45.

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