TRIIODOTHYRONINE (T3) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 6107205

CE

Enzyme Immunoassay for the Quantitative Determination of Triiodothyronine (T3) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

Triiodothyronine (T3) Enzyme Immunoassay.

INTENDED USE

For the quantitative determination of the Triiodothyronine (T3) concentration in human serum.

INTRODUCTION

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last two decades. The advent of monospecific antiserum and the discovery of blocking agents to the T3 binding serum proteins has enabled the development of procedurally simple radioimmunoassays (1,2). This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate is added, then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native triiodothyronine for a limited number of antibody combining sites immobilized on the well. After the completion of the required incubation period, the antibody bound enzyme-triiodothyronine conjugate is separated from the unbound enzyme-triiodothyronine conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known triiodothyronine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with triiodothyronine concentration.

PRINCIPLE OF THE TEST

Competitive Enzyme Immunoassay

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS

Materials provided with the kit:

- Microwell plate. 1x96-well coated with goat anti mouse pAb and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
- Standards. 0.75 mL/vial - Six vials of serum reference for triiodothyronine at concentrations of 0 , 0.5 , 1.0 , 2.5 , 5.0 and 10.0 ng/mL. Store at 2-8°C. A preservative has been added.
- Enzyme Conjugate. (ready to use) 6 mL/vial. It contains triiodothyronine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. Store at 2-8°C.
- Assay Reagent – 6 mL. One bottle containing buffer, binding protein inhibitors and anti T3 mAb. Store at 2-8°C.
- Washing Solution Concentrate (40x). 25 mL, 40x concentrated. A preservative has been added. Store at 2-30°C.
- TMB-Substrate. 1 x 12.0 mL/vial , ready to use. Store at 2-8°C.
- Stop Solution. 1 x 12.0 mL/vial containing a strong acid (0,5 N H_2SO_4). Store at 2-30°C.

Note 1: Do not use reagents beyond the kit expiration date.

PRECAUTIONS

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

Collect sample(s) by venipuncture in ten (10) mL silicone evacuated tube(s) or evacuated tube(s) containing EDTA or heparin. The usual precautions in the collection of venipuncture samples should be observed. Separate the red blood cells by centrifugation, use serum or plasma for the total T3 procedure. Specimen(s) may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) can not be assayed

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within 48 hours, the sample(s) may be stored at temperatures of -20°C for up to 30 days. When assayed in duplicate, 0.10 mL (50 µL per test) of the specimen is required. The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of triiodothyronine needed to displace the same amount of tracer.

Substance	Cross Reactivity (%)
I-Triiodothyronine	100
I-Thyroxine	0.37
Reverse T3	0.75
D-Thyroxine	0.1
3,5-Diiodo-L-Thyrosine	0.2
4-Phenoxyphenol	0.2

Materials Required but not provided

1. Pipette capable of delivering 50µL volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100mL and volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Quality control materials.
7. Vacuum aspirator (optional) for wash steps.
8. Timer

REAGENT PREPARATION

1. *Wash Buffer*. Dilute contents of Wash Concentrate to 1000mL with distilled or deionized water in a suitable storage container. Store at room temperature until expiration date printed on concentrate label.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate.

2. Pipette 50 µL of the appropriate serum reference, (control) or specimen into the assigned well. It is important to add first the standards or sera before adding the assay reagent.

3. Add 50 µL of assay reagent to all wells .

4. Swirl the microplate gently for 10 seconds to mix and cover.

5. Incubate 30 minutes at room temperature (20 – 27°C).

6. Add 50 µL of Triiodothyronine-enzyme conjugate solution to all wells.

7. Swirl the microplate gently for 10 seconds to mix and cover.

8. Incubate 30 minutes at room temperature (20 – 27°C).

9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

10. Add 300 µL of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times.

11. Add 100 µL of TMB-substrate to all wells. Always add reagents in the same order to minimize reaction time differences between wells.

    Incubate 10 minutes at room temperature (20 – 27°C).
    The incubation times are adjusted to 20- 27°C. In case the room temperature is higher than 27°C incubate the TMB-substrate only for 8 minutes (absorption of 0-standard should be not higher than 2.5).

12. Add 100 µL of stop solution to each well. Always add reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 450 nm in a microplate reader.The results should be read within ten minutes of adding the stop solution.

Note: For re-assaying specimens with concentrations more than 10 ng/mL, pipette 25µL of the specimen and 25µL of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the triiodothyronine concentration.

QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from

established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine in unknown specimens.

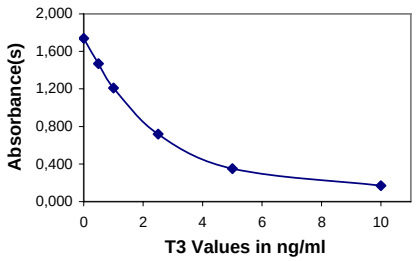
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding T3 concentration in ng/mL on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis (y-axis) of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis (X-axis) of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.208 (intersects the dose response curve at (106 ng/dL) T3 concentration (See Figure 1).

Figure 1



EXAMPLE 1

WELL	SERUM REFERENCES	ABSORBANCE
1	0.0 ng/mL	1.74
2	0.5 ng/mL	1.47
3	1.0 ng/mL	1.21
4	2.5 ng/mL	0.72
5	5.0 ng/mL	0.35
6	10.0 ng/mL	0.17

*The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

Q.C. PARAMETER.

Maximum Absorbance (O calibrator) = 1.3 - 2.5

LIMITATIONS OF PROCEDURE

A. Assay Performance. Serum references and controls should not exhibit cloudiness with time. Discard if cloudiness is observed. Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or hemolysed specimen(s) should similarly not be used. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond 5 minutes to avoid assay drift. If more than 1 plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results. Unused microwell strips should be re-inserted into the aluminum foil bag and re-sealed with the ziploc.

INTERPRETATION

If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of triiodothyronine to TBG (3, 4). Thus, total Triiodothyronine concentration alone is not sufficient to assess clinical status. A decrease in total triiodothyronine 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 total Triiodothyronine values has been compiled by the Journal of the American Association of Clinical Chemists[3].

EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the T3 ELISA Test System. The mean (R) values standard deviations (S.D.) and expected ranges (±2 S.D.) are presented in Table 1. The total number of samples was 105.

TABLE 1

Expected Values for the T3 ELISA Test System
(in ng/mL)

Mean (X)	1,315
Standard Deviation (S.D.)	0,334
Expected Ranges (±2 S. D.)	0.49 – 2.02

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method

for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the T3 Microplate ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2

Within Assay Precision (Values in nmol/L)

Sample	N	X	S.D.	C.V.
Low	24	2.02	0.04	3.52%
Normal	24	4.01	0.02	3.01%
High	24	5.68	0.02	3.73%

TABLE 3

Between Assay Precision* (Values in ng/mL)

	Sample 1	Sample 2	Sample 3
Lot:20730.1			
X	2.02	4.0	5..68
SD	0.04	0.02	0.02
CV	3.52	3.01	3.73
Lot:20802.1			
X	2.13	3.90	5.33
SD	0.05	0.02	0.02
CV	6.49	5.11	4.63
CV[%]	5.16	2.62	6.08

B. Accuracy

The T3 ELISA Test System was compared with a reference radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.15 ng/mL – 8.0 ng/mL). The total number of such specimens was 120. The least square regression equation (y= mx+b) and the correlation coefficient were computed for the T3 ELISA in comparison with the reference method.

C. Sensitivity

The Triiodothyronine test system procedure has a sensitivity of 0.1 ng/mL.

REFERENCES

1. Barker, S.B., "Determination of Protein Bound Iodine." Joumal Biological Chemistry, 173, 175, (1948).
2. Chopra, I.J., Solomon, D.H., an 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).

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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