TOTAL THYROXINE (T4) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 6107215

CE

Enzyme Immunoassay for the Quantitative Determination of Total Thyroxine (T4) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

T4 Enzyme Immunoassay

INTENDED USE

For the quantitative determination of the Total Thyroxine (T4) concentration in human serum.

INTRODUCTION

Measurement of serum thyroxine concentration is generally regarded as an important in-vitro diagnostic test for assessing thyroid function. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last three decades. This procedural evolution can be traced from the empirical protein bound iodine (PBI) test (1) to the theoretically sophisticated radioimmunoassay (2).
The 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-T4 conjugate is added, then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native thyroxine for a limited number of antibody combining sites immobilized on the well.
After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine 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 thyroxine 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 thyroxine 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 insolubulized 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. 12x8 well strips. Individually separable wells. Coated with sheep anti-T4, packaged in an aluminum bag with a drying agent.
- Standards. 6 Vials x 0.2 mL. The concentrations are started on the label. Preservative: Thimerosal 0.01% Kathon 0,1%
- Enzyme Conjugate. T4-horseradish peroxidase (HRP) conjugate. 12 mL..
- Washing Solution Concentrate (40x). Surfactant in phosphate buffered saline. A preservative has been added. 25 mL.
- TMB-Substrate. H_2O_2-TMB 0.25 g/L (avoid any skin contact). 12 mL
- Stop Solution. Sulphuric acid 0.25 M (corrosive: avoid any skin contact). 12 mL

Materials required but not provided:

- Multichannel pipettes and micropipettes (Precision ≥1.5%) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

ASSAY PROCEDURE

1. Prior to use bring all reagents to room temperature.
2. Prior dilutions :

Dilute the washing buffer in 1 L distilled water.

3. Pipette 10 µL of e.g. standards or samples into each well.
   Pipette all standards and samples within 10 minutes !
4. Incubate 5 minutes at room temperature.
5. Add 100 µL peroxidase conjugate into each well.
6. Mix by moving plate on the table (10 sec)
7. Incubate 80 minutes at room temperature (18-26°C)
8. Remove solution from the wells by aspirating or tapping the plate.
9. For washing fill plate with Washing buffer and remove; repeat wash 4x.
10. Pipette 100 µL TMB-substrate solution into each well

11. Incubate 10 min at room temperature (18-25°C)
    Incubate 7 min at room temperature (26-29°C)
    Incubate 5 min at room temperature (more than 29°C)
12. Add 100 µL stopping solution into each well (same order as substrate solution)

Results:

The absorbance of the samples is measured with a conventional ELISA-Reader at 450 nm against air (reference wavelength 620-655 nm).

The concentrations of T4 can be calculated automatically by using an ELISA software or manually by plotting the standard concentrations over their absorbances in a lin-lin plot.
T4 concentrations of the samples can be calculated directly from the standard curve.

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 standard 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.

CALCULATION OF RESULTS

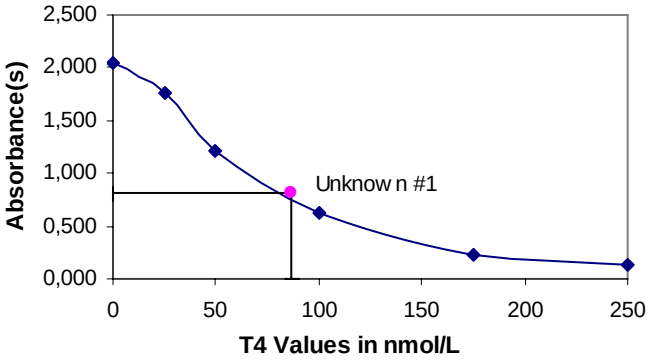
A dose response curve is used to ascertain the oncentration of thyroxine in unknown specimens.

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 T4 concentration in nmol/L 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 T4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in nmol/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 0.82 (intersects the standard curve at (87 nmol/L) T4 concentration (See Figure 1).

EXAMPLE 1

WELL	SERUM REFERENCES	ABSORBANCE
1	0.0 nmol/L	2.06
2	0.0 nmol/L	2.03
3	25 nmol/L	1.78
4	25 nmol/L	1.74
5	50 nmol/L	1.23
6	50 nmol/L	1.19
7	100 nmol/L	0.62
8	100 nmol/L	0.64
9	175 nmol/L	0.25
10	175 nmol/L	0.23
11	250 nmol/L	0.13
12	250 nmol/L	0.12

Figure 1



Well	Unknown I. D.	O.D.	Avg. O.D.	Value
13	Unknown #1	0,81		
14	Unknown #1	0,83	0,82	87 nmol/L

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

Q.C. PARAMETER.

Maximum Absorbance (O calibrator) = 1.7 - 2.9

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 ten (10) minutes to avoid assay drift. If more than one (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.

B. 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 thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG (3, 4). Thus, total thyroxine concentration alone is not sufficient to assess clinical status.Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A T3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated T4 is caused by TBG variation.A decrease in total 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 total thyroxine values has been compiled by the Journal of the American Association of Clinical Chemists.

"NOT INTENDED FOR NEWBORN SCREENING"

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population.

TABLE 1

Expected Values for the T4 ELISA Test System (in nmol/L)

	Male (42 specimens)	Female * (58 specimens)
Mean (X)	76	82
Standard Deviation (S.D.)	1.6	1.7
Expected Ranges (±2 S. D.)	44 – 108	48 – 116

*Normal patients with high TBG levels were not excluded except if pregnant.

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 T4 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.
Pool 1	24	103.65	3.56	3.43
Pool 2	24	145.65	3.64	2.50
Pool 3	24	194.40	11.59	5.60

TABLE 3

Between Assay Precision* (Values in nmol/L)

	Sample 1	Sample 2	Sample 3
Lot:1			
X	93.57	137.2	189.15
SD	3.66	9.55	15.1
CV	3.91	6.96	7.98
Lot:2			
X	103.55	145.65	194.6
SD	3.56	3.64	11.59
CV	3.43	2.48	6.00
Deviation [%]	9.73	5.78	2.80

*As measured in ten experiments in duplicate over a ten day period.

B. Accuracy

The T4 Microplate ELISA Test System was compared with a coated tube radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 8 nmol/L – 250 nmol/L). The total number of such specimens was 131. The least square regression equation and the correlation coefficient were computed for this T4 ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	80.7	y = 0.39+0.952(x)	0.934
Reference	80.6		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity

The thyroxine procedure has a sensitivity of 10 nmol/L. The sensitivity was ascertained by determining the variability of the 0 nmol/L serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

REFERENCES

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QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona, SPAIN
Telf. (+34) 934 694 990 Fax. (+34) 934 693 435. website www.linear.es