

Intended Use

For the quantitative determination of Free Triiodothyronine (fT3) concentration in human serum.

Test Summary

L-Triiodothyronine, a thyroid hormone, circulates in blood almost completely bound (>99.5%) to carrier proteins. The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Furthermore, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In individuals with normal thyroid function, as the concentrations of the carrier proteins change, the total T3 levels also change so that the free triiodothyronine (fT3) concentration remains constant. Thus, measurements of fT3 concentrations correlate more reliably with clinical status than total triiodothyronine levels.

For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives, and estrogen therapy result in higher total T3 levels while fT3 concentration remains basically unchanged.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of fT3.

Principle

The fT3 test is a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and T3-Enzyme Conjugate Working Reagent are added to wells coated with monoclonal T3 antibody. fT3 in the patient specimen and the T3 labeled conjugate compete for available binding sites on the antibody. After a 60 minute incubation at room temperature, the wells are washed with water to remove unbound T3 conjugate. A solution of H₂O₂/TMB is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 3N HCl, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the color formed is 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. T3 Antibody-Coated Microplate, 96 wells
Microtiter wells coated with Anti-T3.
2. T3-Enzyme Conjugate Reagent, ready to use, 10.5ml
Contains T3 Ab. conjugated to horseradish peroxidase with preservatives.
3. Free T3 Reference Standards, 0, 0.9, 2.2, 5.0, 9.0, and 19.0 pg/ml of fT3 in serum with preservatives; liquid, ready to use. *Exact levels are given on the labels on a lot specific basis.
4. Color Reagent A, 13ml. Contains hydrogen peroxide in acetate buffer.
5. Color Reagent B, 13ml. Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
6. Stop Solution (3N HCl), 10ml. Contains dilute hydrochloric acid.

Materials Required but not Provided

1. Pipettes capable of delivering 50µl volumes with precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050ml and 0.200ml volume with a precision of better than 1.5%.
3. Microplate Reader with 450 nm wavelength absorbance capability.

4. Test tubes for dilution of enzyme conjugate and for mixing Color Reagent A with Color Reagent B.
5. Absorbent paper for blotting the microplate wells.
6. Timer.
7. Quality Control materials.

Specimen Collection and Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Serum samples may be refrigerated at 2-8°C for a maximum period of 48 hours. If the samples cannot be assayed within 48 hours, they may be stored at temperatures of -20°C for up to 30 days.

Storage of Test Kit and Instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent Preparation

Working Substrate Solution – Prepare immediately before use

To prepare H₂O₂/TMB solution, make an 1:1 mixing of Color Reagent A with Color Reagent B up to 1 hour before use. Mix gently to ensure complete mixing. The prepared H₂O₂/TMB reagent should be made at least 15 minutes before use and is stable at room temperature in the dark for up to 3 hours. Discard excess after use.

Assay Procedure

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-25°C).

1. Format the microplates' wells for each serum reference, control, and patient specimen to be assayed in duplicate.
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control and specimen into the assigned well.
3. Add 0.100 ml (100µl) of Free T3 Enzyme Conjugate Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 0.200 ml (200µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
8. Incubate at room temperature in the dark for 20 minutes.
9. Stop the reaction by adding 50µl of 3N HCl (Stop Solution) to each well.
10. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
11. Read absorbance at 450 nm with a microtiter well reader within 30 minutes.

Calculations

1. Calculate the mean absorbance value (A₄₅₀) for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/dl on graph paper,

Free Triiodothyronine (fT3) Enzyme Immunoassay Test Kit

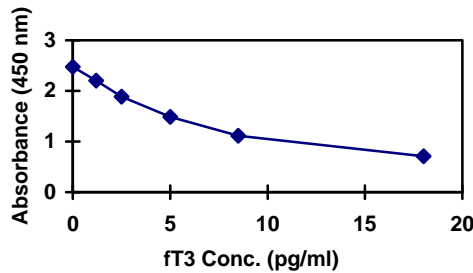
with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.

- Use the mean absorbance values for each specimen to determine the corresponding concentration of fT3 in pg/ml from the standard curve.

Example of Standard Curve

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against fT3 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

FT3 (pg/ml)	Absorbance (450nm)
0	2.474
1.2	2.202
2.5	1.884
5.0	1.485
8.5	1.117
18.0	0.710



Performance Characteristics

1. Accuracy

The fT3 Microplate EIA Test System was compared with a coated tube radioimmunoassay method. Biological specimens from hypothyroid, euthyroid, and hyperthyroid populations were used (Values ranged from 0.1 pg/ml – 14 pg/ml). The total number of such specimens was 85. The least square regression equation and the correlation coefficient were computed for this fT3 EIA Test System in comparison with the reference method. The data obtained is shown in the table below:

Method	Mean(X)	Least Square Regression Analysis	Coefficient
This method	3.045	$y=0.978(x)-0.116$	0.950
Reference	2.921		

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.

2. Precision

The within and between assay precision of the fT3 microplate EIA 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 shown in the following tables:

		Within Assay Precision (Values in pg/ml)		
Sample	N	X	S.D.	C.V.
Low	24	1.85	0.09	4.9%
Normal	24	4.49	0.16	3.6%
High	24	8.00	0.25	3.1%

		Between Assay Precision (Values in pg/ml)*		
Sample	N	X	S.D.	C.V.
Low	12	2.16	0.29	13.1%
Normal	12	5.09	0.40	7.9%
High	12	9.13	0.94	10.2%

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

3. Specificity

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 Triiofthyronine needed to displace the same amount of tracer.

Substance	Cross-Reactant	Concentration
I-Triiodothyronine	1.0000	
I-Thyroxine	<0.0002	10 ug/ml
Iodothyrosine	<0.0001	10 ug/ml
Diiodothyrosine	<0.0001	10 ug/ml
Diiodothyrosine	<0.0001	10 ug/ml
Phenylbutzone	<0.0001	10 ug/ml
Sodium Salicylate	<0.0001	10 ug/ml

4. Sensitivity

The fT3 EIA procedure has a sensitivity of 0.05 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

Expected Values

A study of euthyroid adult population was undertaken to determine expected values for the fT3 EIA Test System. The mean (X) values, standard deviations (S.D.) and expected ranges (± 2 S.D.) are presented in the table below.

Expected Values for the Free T3 EIA Test System (in pg/ml)

	Adult (110 specimens)	Pregnancy (75 specimens)
Mean (X)	2.8	3.0
Standard Deviation	0.7	0.6
Expected Ranges (+ 2 S.D.)	1.4 – 4.2	1.8 – 4.2

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 several 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.

Clinical Significance

Alterations in the concentration of serum binding proteins will generally result in a corresponding change in total T3 concentrations while the physiologically active fT3 level remains largely unchanged in a euthyroid individual. Therefore, determination of fT3 concentration may provide a more accurate assessment of thyroid status than total T3 measurement. Elevated fT3 Concentrations are indicative of hyperthyroidism and low levels are indicative of hypothyroidism.

Limitations

1. 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 food laboratory practice
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, 2nd Ed., pg. 602, Saunders Press, Phila., 1976.
2. Horworth, P.J.N., Ward, R.L., J. Clin. Pathol. 1972; 25:259-62.
3. Sati, C., Chattor, A.J., Watts, N. Fundamentals of Clinical Chemistry. Ed. Tietz, N.W. 3rd Ed., pg. 586. Saunders Press Phila. 1987.
4. Lundberg, P.A., Jagenburg, R., Lindstedt, G., Nystrom, E., Clin. Chem. 1982, 28:1241.
5. Melmed, S., Geola, F.L., Reed, A.W., Pekary, A.E., Park, J., Hershmen, J.M., Clin Endocrin. Metabol. 1982, 54:300.
6. Ingbar, S.H., et al. J. Clin. Invest., 1965, 44:1679.
7. Selenkow, H.A., and Robin N.I., J. Maine Med. Assoc. 1970, 61:199.
8. Oppenheimer, J.H., et al. J. Clin. Invest. 1962, 42:1769.
9. Dick, M., Watson, F., Med. J. Aust. 1980, 1:115.
10. Dussault, J. H., Turcotte, R., and Gieyda, H., Clin. Endocrin. Metabol. 1976, 43:232-285.
11. Tamoky, A.L., Advan. Clin. Chem. 1981, 21:101-146.
12. Emrich, D., Schondube, H., Sehlen, S., and Schreivagel, I., Nuc. Compact, 1985, 16:392.
13. Procedures for Decontamination of Plumbing Systems Containing Copper and/or Lead azides, Dept. of H.E.W., N.I.O.S.H., Rockville, Maryland, 1976.

051707

Manufactured for Pointe Scientific, Inc.
5449 Research Drive, Canton, MI 48188

European Authorized Representative:
Obelis s.a.

Boulevard Général Wahis 53
1030 Brussels, BELGIUM

Tel: (32)2.732.59.54 Fax: (32)2.732.60.03 email: mail@obelis.net



Rev. 10/13 P803-T1006-01