T3 (Free) (Human) ELISA Kit

Catalog Number KA0199

96 assays

Version: 04

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Intended Use

T3 (Free) (Human) ELISA Kit for the quantitative determination of free Triiodothyronine (fT3) concentration in human Serum.

Background

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 change in consert so that the free thriiodothyronine (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 the 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 of the Assay

The fT3 test is a solid phase competitive enzyme immunoassay. Serum samples, standards, and T3-Enzyme Conjugate Working Reagent is added to wells coated with monoclonal T3 antibody. fT3 in the specimen and the T3 labeled conjugate compete for available binding sites on the antibody. After a 60 minutes incubation at room temperature, the wells are washed with water to remove unbound T3 conjugate. A solution of H_2O_2/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 450 nm. 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 condentration of fT3 in the unknown sample is quantified.

General Information

Materials Supplied

List of component

Component	Amount
Antibody-Coated Microplate: Microtitr wells coated with Anti-T3.	96 (8x12) wells
fT3-Enzyme Conjugate Reagent, ready to use: Contain T3 Ab conjugated to horseradish perosidase with preservatives.	10.5 mL
Free T3 Reference Standards Set: In human serum with preservatives, liquid, ready to use. *Note: Exact levels are given on the labels on a lot specific basis.*	1 mL x 6
Color Reagent A Contains hydrogen peroxide in acetate buffer	13 mL
Color Reagent B Contains 3,3',5,5' tetramethylbenzidine (TMB) stabilized in buffer solution.	13 mL
Stop Solution (3N HCl) Contains diluted hydrochloric acid	10 mL

Storage Instruction

Unopened 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.

Materials Required but Not Supplied

- ✓ Pipette capable of delivering 50 µL volumes with a precision of better than 1.5%.
- ✓ Dispenser(s) for repetitive deliveries of 0.050 mL and 0.200 mL volumes with a precision of better than 1.5%.
- ✓ Microplate Reader with 450 nm wavelength absorbance capability. (A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement).
- ✓ Test tubes for dilution of enzyme conjugate and for mixing Color Reagent A with Color Reagent B.
- ✓ Absorbent paper of blotting the microplate wells.
- ✓ Timer.
- ✓ Quality control materials

Precautions for Use

✓ Limitation of procedures

- 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 results in poor precision and falsely elevated absorbance readings.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedure and information available to the physician.

Assay Protocol

Reagent Preparation

Working Substrate Solution – Prepare immediately before use.
To prepare H_2O_2/TMB solution, make a 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 H_2O_2/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.

Sample Preparation

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

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 (50 µL) of the appropriate serum reference, control, and specimen into the assigned well.
3. Add 0.100 mL (100 µL) of T3-Enzyme Conjugate Solution 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 (200 µL) 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. Gently mix for 10 seconds.
8. Incubate at room temperature in the dark for 20 minutes.
9. Stop the reaction by adding 50 µL of 3N HCl 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.

Data Analysis

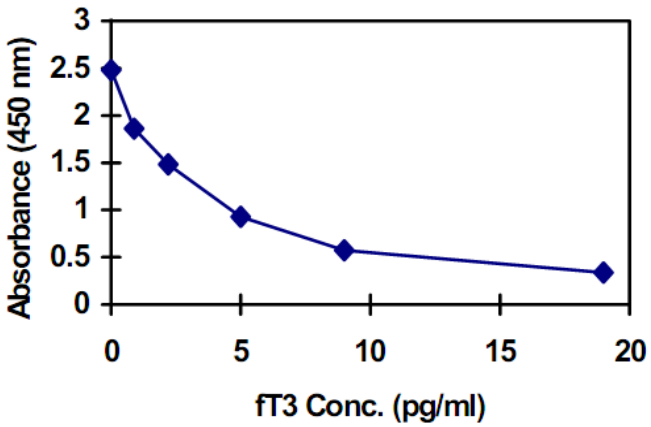
Calculation of Results

- ✓ Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.
- ✓ Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/mL on graph paper, 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 450 nm 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 in each experiment.

fT3 (pg/mL)	Absorbance (450 nm)
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

✓ Accuracy

This ELISA assay 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 151. The least square regression equation and the correlation coefficient were computed for this ELISA assay in comparison with the reference method. The data obtained is shown in the following table:

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.

✓ Precision

The within and between assay precision of this ELISA assay 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.

✓ 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 ration between dose of interfering substance to dose of Triiodothyronine needed to displace the same amount of tracer.

Substance	Cross Reactivity	Concentration
I-Triiodothyronine	1.0000	-
Iodothyrosine	<0.0001	10 µg/mL
Diiodothyrosine	<0.0001	10 µg/mL
Diiodothyrosine	<0.0001	10 µg/mL
Phenylbutzone	<0.0001	10 µg/mL
Sodium Salicylate	<0.0001	10 µg/mL

✓ Sensitivity

This ELISA assay 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 This ELISA assay. The mean (X) values, standard deviations (σ) and expected ranges (± 2σ) are presented in the following table:

Expected Values for the T3 (Free) (Human) ELISA Kit (in pg/mL)		
	Adult (110 specimens)	Pregnancy (75 specimens)
Mean (X)	2.8	3.0
Standard Deviation (σ)	0.7	0.6
Expected Ranges (± 2σ)	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.

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 an 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.

Resources

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, 2nd Ed., pg. 602, Sauders Press, Phila., 1976.
2. Horworth, P.J.N., Ward, RL., 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. Tarnoky, 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.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H