# Free TRIIODOTHYRONINE (fT3)

**ENZYME IMMUNOASSAY TEST KIT**
**Catalog Number: 10305**

## Enzyme Immunoassay for the Quantitative Determination of Free Triiodothyronine (fT-3) In Human Serum

### INTENDED USE

The Quantitative Determination of Free Triiodothyronine Concentration in Human Serum by a Microplate Enzyme Immunoassay. Levels of fT3 are thought to reflect the amount of T3 available to the cells and may therefore determine the clinical metabolic status of T3.

### INTRODUCTION

Triiodothyronine, a thyroid hormone, circulates in blood almost completely bound (>99.5%) to carrier proteins[1,2] . 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. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total triiodothyronine level changes so that the free triiodothyronine concentration remains constant. Thus, measurements of free triiodothyronine 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 free T3 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 free T3. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate (analog method) is added, then the reactants are mixed. A competition reaction results between the enzyme conjugate and the free 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 free 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 free triiodothyronine concentration.

### PRINCIPLE OF THE ASSAY

Competitive Enzyme Immunoassay Analog Method for Free T3. The essential reagents required for a solid phase enzyme immunoassay include immobilized T3 antibody, enzyme-T3 conjugate and native free T3 antigen. The enzyme-T3 conjugate should have no measurable binding to serum proteins especially TBG and albumin. The method achieves this goal.

Upon mixing immobilized antibody, enzyme-T3 conjugate and a serum containing the native free T3 antigen, a competition reaction results between the native free T3 and the enzyme-T3 conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the followed equation:

$$EnzAg + Ag + AbC.W. \underset{K\text{-}a}{\overset{ka}{\rightleftharpoons}} AgAbC.W. + EnzAgAbC.W.$$

AbC.W. = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
EnzAg = Enzyme-antigen Conjugate (Constant Quantity)
AgAbC.W. = Antigen-Antibody Complex
EnzAg AbC.W. = Enzyme-antigen Conjugate -Antibody Complex
ka = Rate Constant of Association
k-a = Rate Constant of Disassociation
K = ka / k-a = Equilibrium Constant

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 free 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.

### STORAGE CONDITIONS

1. Store the kit at 2 to 8°C upon receipt and when it is not in use.
2. Keep microtiter wells in a sealed bag with desiccants.

### INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

### 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

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

### MATERIALS PROVIDED WITH TEST KIT

1.Anti-T3 Antibody-Coated Microtiter Wells, 96 wells.
2.fT3 HRPO Conjugate Reagent, 11 mL
3.Reference Standard,1 set. 1.0 mL/vial,for SI units:1pg/mL x 1.536 = pmol/L.
* Exact levels are given on the labels on a lot specific basis. Ready to use.
4.Wash Solution Concentrate, 20mL, One vial containing a surfactant in phosphate buffered saline. A preservative has been added. Store at 2-30°C.
5.Substrate A, 7.0 mL.
6.Substrate B, 7.0 mL
7.Stop Solution, 6.0mL, One bottle containing a strong acid . Store at 2-30°C.

**Note:**
1.Do not use reagents beyond the kit expiration date.
2.Opened reagents are stable for sixty (60) days when stored at 2-8°C.

### MATERIALS REQUIRED BUT NOT PROVIDED

1.Distilled water.
2.Precision pipettes: 0.05mL, 0.1mL, 0.3mL, 1.0 mL.
3.Disposable pipette tips.
4.Microplate washer or a squeeze bottle (optional).
5.Glass tube or flask for preparing TMB solution.
6.Microtiter well reader.
7.Vortex mixer or equivalent.
8.Absorbent paper.
9.Timer.
10.Quality control materials.

### REAGENT PREPARATION

1.All reagents should be allowed to reach room temperature (18-25°C) before use.
2.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. It is essential that all the contents of the wash buffer concentrate dissolve. Crystal formation in the Wash Concentrate can be eliminated by briefly (approx. 5 minutes) heating in a water bath at 37°C or storing the Wash Concentrate at room temperature.
3.Working Substrate Solution, Prepare daily. Determine the amount of reagent needed and prepare by mixing equal portions of Substrate A and Substrate B in a suitable container. For example, add 1mL of A and 1mL of B per two (2) eight well strips (A slight excess of solution is made). Use within twenty-four hours of preparation for maximum performance of the assay.

### ASSAY PROCEDURE

1.Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C
2.Pipette 0.05 mL (50µL) of the appropriate serum reference, control or specimen into the assigned well.
3.Add 0.1 mL (100µL) of fT3-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.Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7.Add 300µL of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two additional times for a total of three 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 two additional times.
8.Add 0.1 mL (100µL) 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.

9.Incubate at room temperature for fifteen minutes.
10.Add 0.05 mL (50µL) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
11.Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty minutes of adding the stop solution.

## 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.

## RESULTS

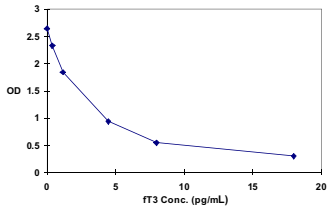
1.A dose response curve is used to ascertain the concentration of free triiodothyronine in unknown specimens.
2.Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
3.Plot the absorbance for each duplicate serum reference versus the corresponding fT3 concentration in pg/mL on linear graph paper (do not average the duplicates of the serum references before plotting).
4.Draw the best-fit curve through the plotted points.
5.To determine the concentration of fT3 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 pg/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.799 (intersects the standard curve at (1.2 pg/mL) fT3 concentration (See Example of Standard Curve).

## EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below.

| FT3 (Pg/mL) | O.D I | 450nm II | Average |
|---|---|---|---|
| 0.0 | 2.639 | 2.615 | 2.627 |
| 0.4 | 2.330 | 2.259 | 2.280 |
| 1.2 | 1.835 | 1.753 | 1.794 |
| 4.5 | 0.939 | 0.975 | 0.957 |
| 8.0 | 0.537 | 0.569 | 0.553 |
| 18.0 | 0.301 | 0.287 | 0.294 |

| I. D. | O.D. | Value |
|---|---|---|
| Unknown #1 | 1.799 | 1.2 pg/mL |




The data presented in here are for illustration only and should not be used in lieu of a standard curve prepared with each assay.
Q.C. PARAMETERS:
Maximum Absorbance (O calibrator) ≥ 1.2.

## LIMITATIONS OF PROCEDURE

**A. Assay Performance**

1.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.
2.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.
3.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.

**B. Interpretation**

1.If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2.Several drugs are known to effect the binding of Triiodothyronine to the thyroid hormone carrier proteins or its metabolism to T3 and complicate the interpretation of free T3 results [3].
3.Circulating autoantibodies to T3 and hormone-binding inhibitors may interfere [4].
4.Heparin has been reported to have in vivo and in vitro effects on free T3 concentration [5]. Therefore, do not obtain samples in which this anti-coagulant has been used.
5.In severe nonthyroidal illness (NTI), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction [6].
6.Familial dysalbuminemic conditions may yield erroneous results on direct free T3 assays [7].

Note: NOT INTENDED FOR NEWBORN SCREENING

## EXPECTED RANGES OF 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 (SD) and expected ranges ± 2 SD are presented in Table 1.

**TABLE I**

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 (SD) | 0.7 | 0.6 |
| Expected Ranges (± 2SD) | 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 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 fT3 Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation (SD) and coefficient of variation (CV) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2: Within Assay Precision (Values in pg/mL)

| Sample | N | X | SD | CV |
|---|---|---|---|---|
| Low | 20 | 1.37 | 0.16 | 11.9% |
| Middle | 20 | 4.21 | 0.17 | 4.9% |
| High | 20 | 7.1 | 0.17 | 2.4% |

TABLE 3: Between Assay Precision (Values in pg/mL)

| Sample | N | X | SD | CV |
|---|---|---|---|---|
| Low | 10 | 1.4 | 0.15 | 10.7% |
| Middle | 10 | 4.4 | 0.23 | 5.2% |
| High | 10 | 7.0 | 0.30 | 4.2% |

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

**B. Accuracy**

The fT3 Microplate EIA Test System was compared with a coated tube radioimmunoassay analog method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.1pg/mL-14pg/mL). The total number of such specimens was 85. The least square regression equation and the correlation coefficient were computed for this fT3 EIA in comparison with the reference method. The data obtained is displayed in Table 4.

**TABLE 4:**

| Method | Mean (x) | Least Square Analysis Regression | Correlation Coefficient |
|---|---|---|---|
| This Method | 3.4 | y = 0.15+0.925(x) | 0.955 |
| Reference | 3.5 | | |

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 Triiodothyronine 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 2 SD (95% certainty) statistic to calculate the minimum dose.

## REFERENCES


1.Pederson, K.O, Scand. J. Clin. Lab Invest, 34, 247 (1974)
2.Wild, D., Immunoassay Handbook, Stockton Press, p339 (1994).
3.Wenzel, K.W., Metabolism, 30, 717 (1981)
4.Bhagat,C.,et.al, Clin Chem, 29, 1324 (1983).
5.Lundberg, P.R., et.al, Clin Chem, 28, 1241 (1982).
6.Melmed, S. et.al, J Clin Endocrinol Metab, 54, 300 (1982).
7.Lalloz M.R., et al, Clin Endocrinol, 18, 11 (1983).


7/2014


**Chemux Bioscience,Inc.**

**Website:** www.chemux.com

385 Oyster Point Blvd Suite5-6.,South San Francisco,CA94080
Tel:+1-650-872-1800