



## TRIIODOTHYRONINE (T3)

ENZYME IMMUNOASSAY TEST KIT  
Catalog Number: 10301

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

#### Intended Use

For the quantitative measurement of total Triiodothyronine (T3) in human serum

#### Introduction

The Human thyroid gland is a major component of the endocrine system. Thyroid hormones perform many important functions. They exert powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance of the body, as well as on the maintenance of metabolic activity and the development of the skeletal and organ system.

The hormones thyroxine (T4) and 3, 5, 3' triiodothyronine (T3) circulate in the bloodstream, mostly bound to the plasma protein, thyroxine binding globulin (TBG). The concentration of T3 is much less than that of T-4, but its metabolic potency is much greater.

T3 determination is an important factor in the diagnosis of thyroid disease. Its measurement has uncovered a variant of hyperthyroidism in thyrotoxic patients with elevated T3 values and normal T4 values. An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. The clinical significance of T3 is also evident in patients in whom euthyroidism is attributable only to normal T3, although their T4 values are subnormal.

T3 determination is also useful in monitoring both patients under treatment for hyperthyroidism and patients who have discontinued anti-thyroid drug therapy. It is especially valuable in distinguishing between euthyroid and hyperthyroid subjects.

In addition to hyperthyroidism, T3 levels are elevated in women who are pregnant, and in women receiving oral contraceptives or estrogen treatment, paralleling TBG increases in a manner analogous to T4 levels. Likewise, a reduction in TBG concentration decreases T3 concentration. These changes in the T3 level, however, are not a true reflection of thyroid status.

#### Principle of the test

In the T3 EIA, a certain amount of anti-T3 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the anti-T3 antibody is bound to the

Second antibody on the wells, and T3 and conjugated T3 compete for the limited binding sites on the anti-T3 antibody. After 60-minute incubation at room temperature, the wells are washed 5 times by water to remove unbound T3 conjugate. A solution of 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 2 N 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 T3 in the sample. By reference to a series of T3 standards assayed in the same way, the concentration of T3 in the unknown sample is quantified.

#### 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 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

#### 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.

#### Materials Provided with Test Kit

- 1.Anti-T3 Antibody-Coated microtiter Wells 96 wells
- 2.T3 HRPO -Conjugate Concentrate 0.8 mL
- 3.T3 HRPO Conjugate Diluent 15 mL
- 4.TMB substrate 12 mL
- 5.Stop Solution 12 mL
- 6.Wash Buffer Concentrate(50X) 15 mL
- 7.Reference standard set,contains 0,0.5,1.0,2.5,5.0,and 10ng/mL of T3, in liquid form(ready to use)or lyophilized form

#### Materials required but not provided

- 1.Distilled water
- 2.Precision pipettes: 40µL~200µL, 200-1000µL, 1.0-5.0 mL
- 3.Disposable pipette tips
- 4.Microtiter well reader
- 5.Vortex mixer or equivalent
- 6.Absorbent paper
- 7.Graph paper

#### Reagent Preparation

- 1.All reagents should be allowed to reach room temperature (18-22°C) before use.
- 2.To prepare T3-HRPO Conjugate Reagent,add 0.1mL of T3-HRPO Conjugate Concentrate to 2.0 mL of T3 Conjugate Diluent (1:20 dilution), and mix well. The amount of conjugate Diluted depends on your assay size. The Conjugate Reagent is stable at 4°C at least for two weeks.

- 3.If reference standards are lyophilized, reconstitute each standard with 0.5mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
- 4.Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15mL of Wash Buffer (50x) into distilled water to prepare 750mL of washing buffer (1x). Mix well before use.

**Note: The T3 assay is a temperature sensitive assay. The best temperature condition for this assay is from 19°C to 22°C. If, in the environmental assay condition, the temperature is higher than expected, we recommend increasing the T3 conjugate dilution (e.g. 1:40) or reducing the incubation time.**

#### Assay Procedure

- 1.Secure the desired number of coated well in the holder. Make data sheet with sample identification.
- 2.Dispense 50µL of standard, samples, and controls into appropriate wells.
- 3.Thoroughly mix for 10 seconds, and then dispense 100µL of enzyme conjugate reagent into each well.
- 4.Thoroughly mix for 30 seconds. It is important to have a complete mixing in this step.
- 5.Incubate at room temperature for 60 minutes.
- 6.Remove the incubation mixture by flicking plate contents into a waste container.
- 7.Rinse and flick the microtiter wells 5 times with washing buffer(1X).
- 8.Strike the wells sharply onto absorbent paper to remove residual water droplets.
- 9.Dispense 100µL TMB solution into each well. Gently mix for 5 seconds.
- 10.Incubate at room temperature in the dark for 20 minutes without shaking.
- 11.Stop the reaction by adding 100µL of Stop Solution to each Well.
- 12.Gently mix for 15 seconds.It is very important to make sure that the blue color changes to yellow color completely.
- 13.Read OD at 450nm with a microtiter reader within 15 Minutes.

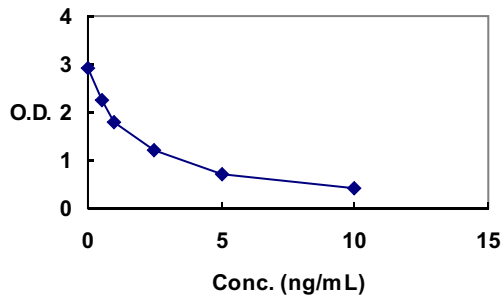
#### Calculation of Results

- 1.Calculate the average absorbance values (A450) for reference standards, control, and samples.
- 2.We recommend using proper software to calculate the results. If the software is not available, construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper,with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- 3.Using the mean absorbance value for each sample,determine the corresponding concentration of T3 in ng/mL from the standard curve.

### Example of Standard Curve

Results of a typical standard run are shown below.

T3 (Ng/mL)	O.D 450 nm		
	I	II	Average
0.0	2.91	2.83	2.87
0.5	2.23	2.22	2.22
1.0	1.80	1.74	1.158
2.5	1.20	1.12	1.16
5.0	0.70	0.66	0.68
10.0	0.41	0.32	0.37



Note: this standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve.

### Expected Values and Sensitivity

Normal Range: 0.6~ 2.0 ng/mL

The minimal detectable concentration of T3 by this assay is estimated to be 0.25 ng/mL.

### Performance characteristics

- Accuracy: Comparison between Our Kits and commercial Available Kits provide the following data  
N = 128  
Correlation Coefficient = 0.977  
Slope = 0.833  
Intercept = 0.012  
Mean (Our Assay) = 0.58  
Mean (Abbott) = 0.62

- Precision
  - Intra-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level I	20	0.79	0.086	10.88
Level II	20	2.54	0.11	4.26
Level II	20	6.45	0.27	4.10

- Inter-Assay

Concentrations	Replicates	Mean	S.D.	% CV
Level I	20	0.68	0.079	11.61
Level II	20	2.11	0.13	5.97
Level II	20	5.73	0.34	6.01

- Linearity:

Two patient sera were serially diluted with 0 ng/mL standard in a linearity study. The average recovery was 100.5 %.

Sample A			
Dilution	Expected	Observed	% Recov.
Undiluted	8.41	8.41	
2x	4.21	3.93	93.5
4x	2.11	2.09	99.3
8x	1.06	1.07	100.9
16x	0.53	0.54	101.9
Average Recovery: 98.9 %			

Sample B			
Dilution	Expected	Observed	% Recov.
Undiluted	8.47	8.47	
2x	4.24	4.04	95.4
4x	2.12	2.23	105.2
8x	1.06	1.14	107.5
16x	0.53	0.53	100.0
Average Recovery: 102.0 %			

- Recovery

Various patient serum samples of known T3 levels were mixed and assayed in duplicate. The average recovery was 95.4 %.

Expected Concentration	Observed Concentration	% Recovery
8.44	9.65	114.3
3.99	3.95	99.0
2.16	1.87	86.6
1.11	0.91	82.0
0.54	0.51	95.3
Average Recovery: 95.4 %		

- Sensitivity:

The sensitivity is defined as the concentration of T3 that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the zero calibrator. The minimum detectable Concentration of this Assay is estimated to be 0.25 ng/mL.

- Cross-reactivity:

The following substances were tested for crossreactivity of The assay:

Antigens	Concentration	Equivalent T3	%Cross-Reactivity
Triiodothyroacetic Acid	100 ng/mL	8.0 ng/mL	8.0
Monoiodothyrosine	50,000 ng/mL	0.45 ng/mL	0.0009
Diiodothyrosine	50,000 ng/mL	0.36 ng/mL	0.0007
Methimazole	50,000 ng/mL	0.31 ng/mL	0.0006
5,5'-Diphenylhydantoin	10,000 ng/mL	0.32 ng/mL	0.0032
Phenylbutazone	1,000,000ng/mL	0.68 ng/mL	0.0007
6-n-propyl-2-thiouracil	100,000 ng/mL	0.65 ng/mL	0.0007
Salicylic Acid	1,000,000ng/mL	0.39 ng/mL	0.0004
Acetylsalicylic Acid	500,000ng/mL	0.38 ng/mL	0.0008

### Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

- As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.
- The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

### References

- Walker W.H.C. Introduction: An Approach to Immunoassay. **Clin. Chem.** 1977; 23: 384
- Kirkegaard C., Friis T. And Siersback-Nielsen K. **Acta Endocrinol.** 1974; 77: 71
- Wisdom G.B. Enzyme-Immunoassay. **Clin. Chem.** 1976; 22: 1243
- Hoffenberg R. **Medicine** 1978; 8: 392
- Lieblich J., Utiger R.D. **J. Clin. Invest.** 1972; 51: 1939

7/2014

### Chemux Bioscience, Inc.

Website: [www.chemux.com](http://www.chemux.com)

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