



## THYROXINE (T4)

### ENZYME IMMUNOASSAY TEST KIT Catalog Number: 10302

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

##### Intended use

For the quantitative determination of thyroxine (T4) in human serum

##### Introduction

L-Thyroxine (T4) is a hormone that is synthesized and stored in the thyroid gland. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of T4 is reversibly bound to three plasma proteins in blood - thyroxine binding globulin (TBG) binds 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%. Approximately 0.03% of T4 is in the free, unbound state in blood at any one time.

Diseases affecting thyroid function may present a wide array of confusing symptoms. Measurement of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in patients. Increased levels of T4 have been found in hyperthyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease), and with some genetic abnormalities.

##### Principle of the test

In the T4 EIA, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, T4 and conjugated T4 compete for the limited binding sites on the anti-T4 antibody. After 60-minute incubation at room temperature,

the wells are washed 5 times by water to remove unbound T4 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 T4 in the sample. By reference to a series of T4 standards assayed in the same way, the concentration of T4 in the unknown sample is quantified.

##### Materials and components

###### Materials provided with the test kits:

- Antibody-coated microtiter wells 96 wells
- T4 HRPO Conjugate Diluent 15 mL
- T4 HRPO Conjugate- Concentrate (20X) 0.8 mL
- TMB Substrate 12 mL
- Stop Solution 12 mL
- Reference standard set, contains 0, 1.0, 2.5, 5.0, 15, 30µg/dL Of T4, in liquid form (ready to use) or lyophilized form
- Wash Buffer Concentrate(50X), 15 mL

###### Materials required but not provided:

- Precision pipettes: 40µL~200µL, 1.0-5.0 mL
- Disposable pipette tips
- Distilled water
- Vortex mixer or equivalent
- Absorbent paper or paper towel
- Graph paper
- Microtiter well reader

##### 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.

##### Storage of test kits and instrumentation

- 1.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. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
- 2.Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

3.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

- 1.All reagents should be brought to room temperature (18-22°C) before use.
- 2.To prepare T4-HRPO Conjugate Reagent, add 0.1 mL of T4-HRPO Conjugate Concentrate to 2.0 mL of T4 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 0.5 mL 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 15 mL of Wash Buffer concentrate(50x) into distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

**Note: The T4 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 T4 conjugate dilution up to 1:40.**

##### Assay procedures

- 1.Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
- 2.Dispense 50µL of standard, specimens, and controls into appropriate wells.
- 3.Dispense 100µL of Enzyme Conjugate Reagent into each well.
- 4.Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this step.
- 5.Incubate at room temperature (18-22°C) 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 or paper towels to remove all residual water droplets.
9. Dispense 100µL of TMB substrate into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes without shaking.
11. Stop the reaction by adding 100µL of Stop Solution to each well.
12. Gently mix for 5 seconds.
13. Read optical density at 450nm with a microtiter well reader.

#### Important Note

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

#### Calculation of results

1. Calculate the average absorbance values ( $A_{450}$ ) for each set of referenc 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 µg/dL 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 T4 in µg/dL from the standard curve.

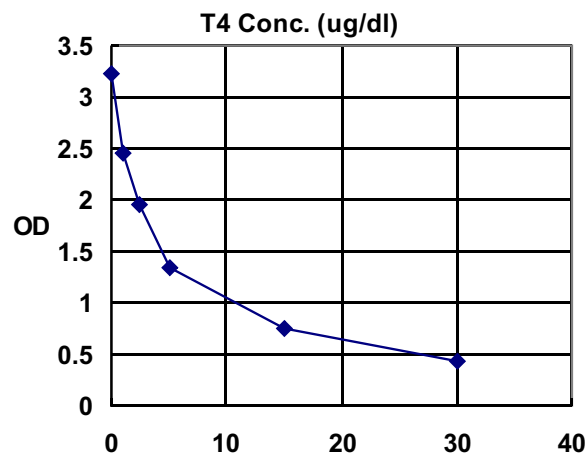
#### Example of standard curve

Results of typical standard run with optical density reading at 450nm shown in the Y axis against T4 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.

T4 (µg/dL)	Absorbance (450nm)
0.0	3.217
1.0	2.465
2.5	1.961
5.0	1.331
15.0	0.746
30.0	0.436

#### Expected values and sensitivity

T4 EIA was performed in a study of 200 euthyroid patients in one geographic location and yielded a normal of 5.0 to 13.0 µg/dL. This range corresponds to those suggested by other commercial manufacturers. It is recommended that laboratories adjust values to reflect geographic and population differences specific to the patients they serve. The minimum detectable concentration of thyroxine by this assay is estimated to be 0.5µg/dL.



#### Limitations of the Procedure

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

- 1) 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.

2) 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.

3) 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

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