

TOTAL THYROXINE (T4) ELISA Kit Protocol

(Cat. No.:EK-310-07)



PHOENIX PHARMACEUTICALS, INC.

INTENDED USE

For the quantitative determination of Total Thyroxine (T4) concentration in human serum. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

LIMITATIONS OF THE PROCEDURE

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 good 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.

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INTRODUCTION AND PROTOCOL OVERVIEW

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. Increased levels of T4 have been found in hyper-thyroidism 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.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

PRINCIPLE OF THE TEST

In the T4 EIA, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of 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 a 60 minutes incubation at room temperature, the wells are washed 5 times by water to remove unbound T4 conjugate. A solution of TMB Reagent is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of Stop Solution, 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 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.

STORAGE

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 450 nm wavelength is acceptable for use in absorbance measurement. **DO NOT FREEZE.**

LIST OF COMPONENTS

Materials Provided with the Kit:

- Sheep anti-T4 coated microtiter wells, 96 wells.
- T4 Reference Standards: 0, 2, 5, 10, 15, and 25 ug/dl, 1 set, 1.0 ml, ready to use.
- Enzyme Conjugate Concentrate (11x), 1.3 ml.
- Enzyme Conjugate Diluent, 13 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials Required but not provided:

- Precision pipettes: 25 µl, 100 µl and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate 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.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare **Working T4-HRPO Conjugate Reagent**, add 0.1 ml of T4-HRPO Conjugate Concentrate (11x) to 1.0 ml of T4 Conjugate Diluent (1:10 dilution), and mix well. *Note: Prepare only the amount of Conjugate that is required each time. Working Conjugate Reagent should be used within 24 hours. Discard the excess after use.*

ASSAY PROCEDURE

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

1. Secure the desired number of coated wells in the holder.
2. Pipette 25 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of **Working Conjugate Reagent** into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°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 distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
13. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

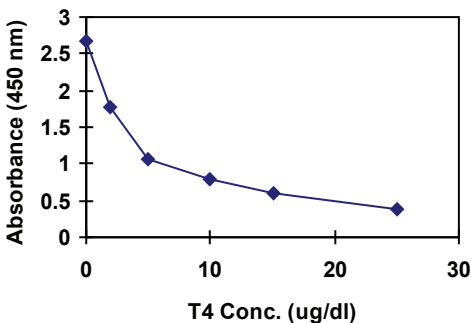
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ug/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 ug/dl from the standard curve.

Each laboratory must provide its own data & std. curve in each experiment.

T4 (ug/dl)	Absorbance (450nm)
0	2.667
2	1.786
5	1.060
10	0.778
15	0.591
25	0.384

Results of a typical standard run with optical density readings at 450 nm 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. 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 in each experiment.



EXPECTED VALUES AND SENSITIVITY

The T4 EIA was performed in a study of 200 euthyroid subjects in one geographic location and yielded a range of 4.8 to 12.0 µg/dl. It is recommended that laboratories adjust values to reflect geographic and population differences specific to the subjects they serve. The minimum detectable concentration of thyroxine by this assay is estimated to be 0.4 µg/dl.

REFERENCES

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