

**TRIIODOTHYRONINE (T3)
ELISA Kit Protocol**

(Cat. No.:EK-310-05)

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INTENDED USE

For the quantitative determination of the Triiodothyronine (T3) concentration in human serum. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

WARNINGS AND PRECAUTIONS FOR USERS

Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984) (22).

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

The thyroid gland exerts powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance, 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 blood stream, mostly bound to the plasma protein, thyroxine binding globulin. (TBG). The concentration of T3 is much less than that of T4, but its metabolic potency is much greater.

T3 determination is an important factor in the diagnosis of thyroid disease. It's measurement has uncovered a variant of hyperthyroidism in thyrotoxic patients with elevated T3 levels and normal T4 levels. An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. In other patients, euthyroidism is attributable to normal T3, although their T4 values are subnormal.

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

In women, T3 levels are elevated during pregnancy, during estrogen treatment, and contraceptive hormone therapy. When T3 levels parallel TBG increases in a manner analogous to T4 levels, these changes are not a reflection of altered thyroid status.

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 T3 EIA, a second antibody (goat anti-mouse IgG) is coated on microtiter wells. A measured amount of patient serum, a certain amount of mouse monoclonal anti-T3 antibody, and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the mouse 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 a 60-minute incubation at room temperature, the wells are washed 5 times by water to remove unbound T3 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 T3 standards assayed in the same way, the concentration of T3 in the unknown sample is then calculated.

STORAGE

Unopened test kits should be stored at 4°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:

- Goat Anti-Mouse IgG Coated Microtiter Wells, 96 wells
- Enzyme Conjugate Concentrate (11x), 1.3 ml.
- Enzyme Conjugate Diluent, 13 ml.
- T3 Reference Standards, 0, 0.75, 1.5, 3.0, 6.0 and 10.0 ng/ml, 1 set, 1.0 ml each, ready to use.
- Antibody Reagent, 7 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml

Materials Required but not provided:

- Precision pipettes: 50 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 T3-HRPO Conjugate Reagent**, add 0.1 ml of T3-HRPO conjugate Concentrate (11x) to 1.0 ml of T3 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 well in the holder. Make data sheet with sample identification.
2. Pipette 50 µl of standard, samples, and controls into appropriate wells.
3. Dispense 50 µl of the Antibody Reagent into each well. Mix thoroughly for 30 seconds.
4. Add 100 µl of **Working Conjugate Reagent** into each well. Mix thoroughly 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 distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 100 µl TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes without shaking.
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 the blue color changes to yellow color completely.***
13. Read OD at 450 nm with a microtiter reader **within 15 minutes.**

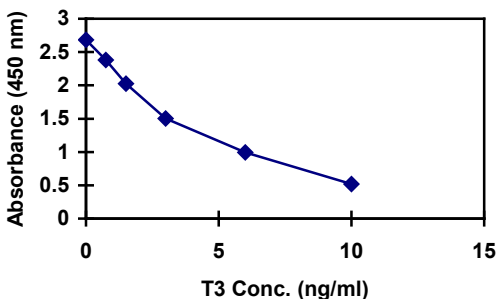
CALCULATION OF RESULTS

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 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.

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

T3 (ng/ml)	Absorbance (450 nm)
0.0	2.685
0.75	2.381
1.5	2.028
3.0	1.502
6.0	0.992
10.0	0.518

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against T3 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.



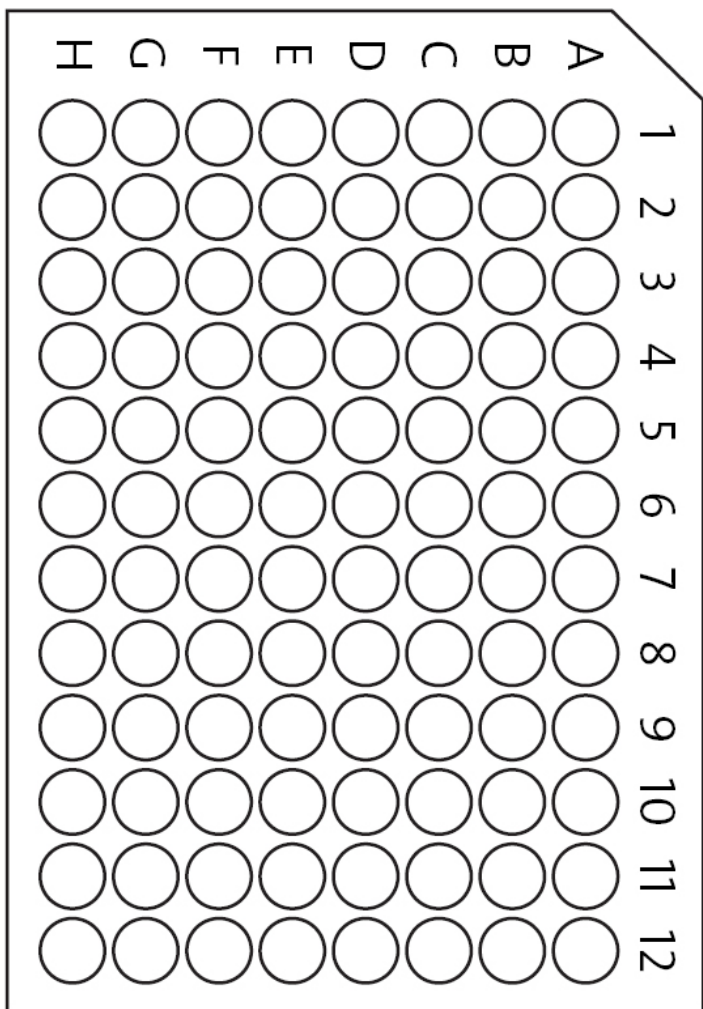
EXPECTED VALUES AND SENSITIVITY

The range in normal individuals is 0.6 – 1.85 ng/ml. In general, total serum T3 levels will tend to parallel the variations in serum levels of the major binding protein, thyroxine-binding globulin (TBG). Elevated T3 levels may be encountered in hypothyroid individuals receiving replacement therapy. The minimum detectable concentration of T3 by this assay is estimated to be 0.2 ng/ml.

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ASSAY DIAGRAM



NOTES