

### T3 ELISA KIT

#### 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 determinations are 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

In the T3 EIA, a certain amount of T3 analog is coated on microtiter wells. A measured amount of patient serum, and a constant amount of anti-T3 antibody conjugated with horseradish peroxidase are added to the microtiter wells. 8 anilino-1-naphthalene-sulfonate (ANS) is used to displace T3 from proteins to enable the measurement of total circulating T3. The reactants are incubated at 37°C. During the incubation T3 analog on microtiter wells and T3 present in the sample, standard compete for binding to the anti-T3 monoclonal antibody-horseradish peroxidase

conjugate. After a 60 minutes incubation at 37°C, the wells are washed 5 times by wash buffer to remove unbound anti-T3-antibody conjugate. Substrate solution and chromogen solution 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 450 nm. The intensity of the color developed is inversely proportional, within the working range of the assay, to the concentration of T3 in the sample. The concentration of T3 in a patient sample or control is then determined by interpolation from the standard curve.

#### MATERIALS

##### MATERIALS PROVIDED

- T3 Analog-Coated Microtiter Wells, 96 wells.
- T3-antibody HRPO Conjugate Concentrate(20x), 1.2 ml.
- T3-antibody HRPO Conjugate Diluent, 15 ml
- Reference Standard, 1 set. contains 0, 0.5, 1.0, 2.5, 5.0 and 10.0 ng/ml. Ready to use..
- Chromogen Solution A 7.5 ml
- Chromogen Solution B 7.5ml
- Stop Solution , 7.5 ml
- Wash buffer concentrate 25ml (40X).

##### MATERIALS NEEDED BUT NOT PROVIDED

- Distilled water.
- Precision pipettes: 0.05ml, 0.1ml, 0.2ml
- Disposable pipette tips.
- Microtiter well reader.
- Vortex mixer or equivalent.
- Absorbent paper.
- Graph paper.

##### STORAGE AND INSTRUMENTATION

- 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.
- Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
- 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

- All reagents should be allowed to reach room temperature ( 18-25°C ) before use.
- To prepare T3-antibody HRPO Conjugate Reagent, add 0.1 ml of T3-HRPO Conjugate Concentrate to 2.0 ml of T3 Conjugate Diluent (1:20 dilution), and mix well. The amount of conjugate diluted is depend on your assay size. The Conjugate Reagent is stable at 4°C at least for 7 days.
- To prepare Washing Buffer: add 1ml of Wash buffer concentration to 39ml of distilled water, and mix well. The washing buffer is stable at R.T. at least for one weeks.
- **Store at 2-8c until the expiration date indicated on the vial label. Once opened the reagents are stable for 2 months and be frozen at -20c for long term storage.**

##### SPECIMEN COLLECTION AND PREPARATION

- Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
- Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
- Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

##### PROCEDURE

1. Secure the desired number of coated well in the holder. Make data sheet with sample identification.
2. Dispense 50 µl of standard, samples, and controls into appropriate wells.
3. Thoroughly mix for 10 seconds, then dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
5. Incubate at 37°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 washer buffer .
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 50 µl Chromogen Solution A into each well. Gently mix for 5 seconds.
10. Dispense 50 µl Chromogen Solution B into each well. Gently mix for 5 seconds
11. Incubate at room temperture in the dark for 20 minutes without shaking.

12. Stop the reaction by adding 50µl of Stop Solution to each well.
13. Gently mix for 15 seconds.
  
14. **It is very important to make sure that the blue color changes to yellow color completely.**
15. Read OD at 450nm with a microtiter reader within 15 minutes.

#### CALCULATION

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. We recommend to use a 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

1. Results of a typical standard run are shown below.

T3(ng/ml)	O.D 450 nm
0.0	2.91
0.5	1.972
1.0	1.347
2.5	0.671
5.0	0.225
10.0	0.085

2. Standard Curve:

#### 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: 0.8~ 1.90 ng/ml

The minimal detectable concentration of T3 by this assay is estimated to be 0.2 ng/ml

#### LIMITATIONS

As with all immunoassays, the results of this test can be influenced by factors present in some patients' specimens. The reagents for this assay have been formulated to minimise interference from heterophilic antibodies and from nonspecific protein binding. However individual sample results may be affected.

For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings. Procedural directions must be followed exactly as any modification of the procedure may change the results. Use of reagents, disposables or spare parts other than those supplied by authorized distributor may produce incorrect results.

Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop human anti-mouse antibodies (HAMA). HAMA can produce either falsely high or falsely low values in immunoassays which use monoclonal antibodies. Samples containing HAMA should not be assayed with the Total T3 assay.

#### REFERENCES

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