CE

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IVD	See external label	2°C-8°C	Σ=96 tests	REF Cat # 3144Z

MICROWELL ELISA
TRIIODOTHYRONINE (T3)
ENZYME IMMUNOASSAY
TEST KIT

T3

Cat # 3144Z

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

Test	**T3 ELISA**
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**Competitive ELISA**
Detection Range	**0-10 ng/mL**
Sample	**50µL**
Specificity	**96.30%**
Sensitivity	**0.2ng/mL**
Total Time	**~90 min**
Shelf Life	**12 months**

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

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' thiiodothyronine (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 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 hyperthroidism and patients who havediscontinued 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 a 60 minutes 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. Reference Standard, 1 set. Ready to use.
5. TMB Substrate, 12 ml
6. Stop Solution ,12 ml
7. Wash Buffer Concentrate (50X),15ml

Materials Required but not Provided

1. Distilled water.
2. Precision pipettes: 0.05~ 0.2ml,1.0ml
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. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.
3. To prepare T3-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 two weeks.
   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 up to 1:40

Assay 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 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 100 µl TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperture 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 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.

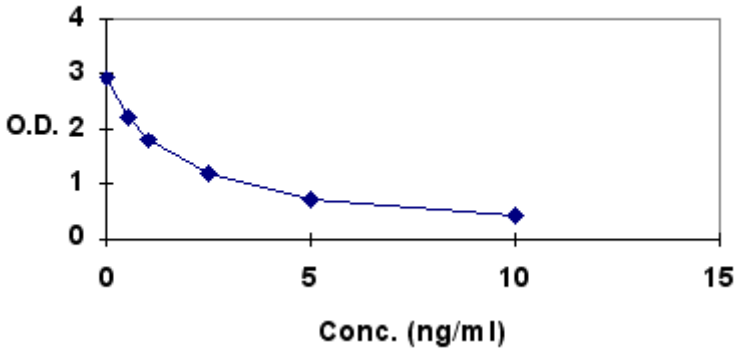
Example of Standard Curve

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

T3	O.D 450 nm		
(ng/ml)	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	1.77
2.5	1.20	1.12	1.16
5.0	0.70	0.66	0.68
10.0	0.41	0.32	0.37

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~ 2.0 ng/ml
The minimal detectable concentration of T3 by this assay is estimated to be 0.2 ng/ml

References

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