Human T-4 (Total Thyroxine) ELISA Kit

Cat. No.:DEIA2266
Pkg.Size:96T

Intended use

For the quantitative determination of total thyroxine (T4) concentration in human serum. The test is useful in the diagnosis and treatment of thyroid disorders.

General Description

The thyroid hormones thyroxine (T4) and triiodothyronine (T3), are synthesized and stored in the thyroid gland and circulate in the bloodstream mostly bound to the plasma protein, thyroxine binding globulin (TBG). The thyroid gland and associated hormones are a major component of the endocrine system. They exert powerful and essential regulatory influences on growth, differentiation, cellular metabolism, and general hormonal balance of the body. 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, TSH, Free T3 and Free T4 by immunoassay are reliable and convenient methods 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 thryoiditis (Hashimoto's disease), and with some genetic abnormalities.

Principle Of The Test

To measure T4 by competitive immunoassay techniques, a sample of serum or plasma containing the T4 to be quantified is mixed with labeled T4 and T4 antibody. The labeled T4 contains 8-anilino-1-napthalene sulfonic acid (ANS) to inhibit binding of T4 to serum proteins, which would otherwise interfere with the assay. During incubation, a fixed amount of labeled T4 competes with the unlabeled T4 in the sample, standard, or quality control serum for a fixed number of binding sites on the specific T4 antibody.
Separation of the unbound T4 from antibody-bound T4 and the subsequent measurement of the labeled fraction of the bound phase completes the test. By comparing results of the unknown sample with those obtained from a series of T4 calibrators, an accurate measurement of the T4 concentration in the sample can be obtained.
In the T4 ELISA, antibody to T4 is coated on a solid phase (microtiter well). A measured amount of patient serum and a constant amount of T4 labeled with horseradish peroxidase are added. During incubation, T4 in the patient sample and enzyme-labeled T4 compete for the limited binding sites on the T4 antibody. After a 60-minute incubation at room temperature, the solid phase is washed with water to remove unbound-labeled T4. A solution of tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color 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 T4 in the patient sample. By reference to a series of calibrators processed in the same way, the concentration of T4 in the unknown sample is determined.

Reagents And Materials Provided

1. Antibody-Coated Wells (1 plate, 96 wells)
Microtiter wells coated with sheep anti-T4.

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2. Enzyme Conjugate Concentrate 11X (1.3 mL)
Contains T4-HRP Conjugate
3. Enzyme Conjugate Diluent (1 bottle, 13 mL)
Contains ANS, TRIS buffer, pH=7.60 and Proclin-300.
4. Reference Standard Set (1 mL/vial)
Contains 0, 2.0, 5.0, 10.0, 15.0 and 25.0 µg/dL in T3/T4-free stripped human serum,
1 set, liquid, ready-to-use.
5. TMB Reagent (1 bottle, 11 mL)
Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
6. Stop Solution (1N HCl) (1 bottle, 11 mL)
Contains diluted hydrochloric acid.

Materials Required But Not Supplied

1. Distilled or deionized water
2. Precision pipettes: 25 µL, 100 µL, 200 µL, and 1 mL
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Absorbent paper
6. Graph paper
7. Vortex mixer or equivalent
8. Quality control material

Storage

1. Store the unopened kit at 2 °C – 8 °C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2 °C – 8 °C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

Specimen Collection And Handling

1. Serum is the sample of choice. Blood should be drawn using standard venipuncture technique and the serum should be separated from the red cells as soon as practical. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with the test procedure.
3. Specimens should be capped and may be stored for up to 48 hours at 2 °C – 8 °C prior to assaying. Specimens held for a longer time (up to 6 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18 °C – 25 °C) before use, and should be mixed by gentle inversion or swirling. Do not induce foaming.
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.
The amount of conjugate diluted depends on the assay size.
The Working Conjugate Reagent is stable at 4 °C for 24 hours.

Assay Steps

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1. Secure the desired number of coated wells in the holder.
2. Pipette 25 µL of standards, specimens, and controls into appropriate wells.
3. Add 100 µL of Working Conjugate Reagent into each well.
4. Mix thoroughly for 30 seconds.
5. Incubate at room temperature (18 °C – 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 H2O.
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 5 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. Ensure that all of the blue color changes completely to yellow.
13. Read absorbance at 450 nm with a microtiter plate reader within 15 minutes.

Quality Control

Good laboratory practice requires that low, medium and high quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

Calculation

1. Calculate the mean absorbance value (OD 450 nm) from the duplicate set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on log-log 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. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

Calibration of Assay

Typical Standard Curve

Results of a typical standard run with optical density readings at 450 nm shown on the Y-axis against Total T4 concentrations (µg/dL) shown on the X-axis, are presented below. NOTE: the standard curve is for illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve for each assay run. Additionally, the absorbance (450 nm) values can be varied due to incubation at different room temperature in different laboratories.

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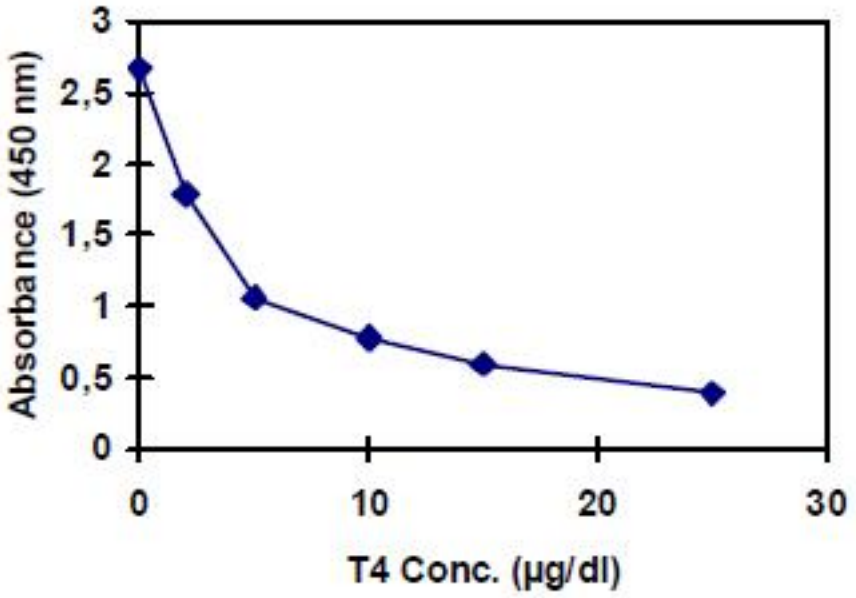
Total T4 (µg/dL)	Absorbance (450 nm)
0	2.667
2	1.786
5	1.060
10	0.778
15	0.591
25	0.384



Reference Values

Expected Values

The T4 ELISA was utilized in a study of 200 euthyroid patient samples (as determined by hospital laboratory analysis) in one geographic l°Cation and yielded a normal range of 5.0 to 13.0 µg/dL. The range was determined by the observed values and corresponds to those suggested by other commercial manufacturers.

It is recommended that laboratories adjust values to reflect geographic and population differences.

Sensitivity

The minimum detectable concentration of T4 that can be defined by this assay is 0.5 µg/dL.

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Specificity

No cross-reactivity.

Linearity

Two samples were serially diluted with T3/T4-free human serum to determine linearity. The mean recovery was 98.8%.

Recovery

Various patient serum samples of known T4 levels were combined and assayed in duplicate. The mean recovery was 96.7%.

Reproducibility

a. Intra-Assay Precision
Within-run precision was determined by replicate determinations of three different control sera in 1 assay.
b. Inter-Assay Precision
Between-run precision was determined by replicate measurements of three different serum samples in several different assays.

Serum Sample	1	2	3
Number of Replicates	26	26	26
Mean T4 (µg/dL)	3.95	8.65	20.51
Standard Deviation	0.17	0.27	0.80
Coefficient of Variation (%)	4.3%	3.1%	3.9%

Serum Sample	1	2	3
Number of Replicates	26	26	26
Mean T4 (µg/dL)	4.29	8.71	19.01
Standard Deviation	0.19	0.35	0.45
Coefficient of Variation (%)	4.5%	4.0%	2.4%

Precautions

1. CAUTION: This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

Limitations

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with good laboratory practice and

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adherence to the package insert instructions.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. Serum samples with T4 concentrations greater than 25 µg/dL should be reported as such. If further quantitation is desired, the sample should be diluted with the Zero Standard and re-assayed. The obtained value should then be multiplied by the dilution factor to obtain the true serum value.
4. Icteric samples with bilirubin values as high as 5 mg/dl do not affect the assay. Additionally, added hemoglobin levels of up to 100 mg/dl showed no effect on the T4 value.
5. Total serum T4 values may be influenced by a variety of factors other than thyroid malfunction. High TSH levels, pregnancy, estrogen therapy, oral contraceptives, heparin, phenytoin and propanolol may all produce invalid results.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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