TOTAL THYROXINE (TOTAL T4)

ELISA KIT Cat. No. KAD-52855

For Quantitative Determination of Total T4 In Human Serum

For In Vitro Research Use Only

ELISA KIT Cat. No. KAD-52855 (96 tests)
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Kit Components (96 tests)
Anti-T4 Coated Strip plate, (96 wells)
T4 Standard A, 0.5 ml; 0 ug/dL
T4 Standard B, 0.5 ml; 2.0 ug/dL
T4 Standard C, 0.5 ml; 5.0 ug/dL
T4 Standard D, 0.5 ml; 10.0 ug/dL
T4 Standard E, 0.5 ml; 15.0 ug/dL
T4 Standard F, 0.5 ml; 25.0 ug/dL
Conjugate Buffer, 12 ml
T4-HRP Conjugate conc. (11X), 1.5 ml
HRP Substrate Solution, 12 ml
Wash Buffer (20X); 25 ml, dilute with 475 ml of distilled water
Stop Solution, 12 ml
Complete Instruction Manual

Introduction

The thyroid gland produces T4, trilodothyronine T3 and calcitonin. The first two hormones are synthesized by the gland following entrapment of iodine, conversion to iodine, and coupling of iodine with tyrosine, followed by coupling of two iodinated tyrosine molecules. T4 and T3 so formed are attached to thyroglobulin for storage and are released, as needed, as protease splits them from the globulin. Thyroxine is a highly active thyrometabolic hormone, which exists, in protein-bound and unbound forms. For T4 can be measured more easily and with greater accuracy than T3, determination of total T4 by immunoassay is the most reliable and convenient screening test available for detecting thyroid disorders in man. Release of T4 and T3 from the thyroid is greatly influenced by pituitary-thyroid stimulating hormone (TSH), which in turn is influenced by hypothalamic thyrotropin-releasing hormone (TRH). Normally, increased blood levels of T4 and T3 act to decrease the amount of TSH secreted, thereby reducing the production and release of T4 and T3. Decreased blood levels of T4 and T3 produce the opposite effect, leading to increased production and secretion of T4 and T3. In this manner a normal circulating thyroid hormone balance is maintained, circulating T4 and T3 are bound largely to thyroxine binding globulin (TBG). To a lesser extent they are bound to thyroxine binding prealbumin (TBPA) and, when present in excess, to albumin. Usually T4 and T3 concentration ratio is about 9:1, however T3 has considerably greater physiological activity. It is the small free fraction (0.1% of the total or less) that is physiologically active and determines the clinical thyroid status of the patient’s hyperthyroid, euthyroid, or hypothyroid.

Inter-assay precision:
Three serum samples (4-15 ug/dL) were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (2.7-4 % CV).

3. RECOVERY
A known amount of total T4 (3-24 ug/dL) was added to three patient sera (with original total T4 concentrations of 3 and 6 ug/dL) and the final total T4 concentration measured. The assay showed good mean recoveries of about 93% (range 85-96%).

4. LINEARITY
A serum sample containing 24 ug/dL was diluted with a series of T4-free serum. The dilutions were tested and the T4 recoveries were compared with the expected concentration. The samples showed excellent mean recoveries of about 102% (range 90-109%).

5. SPECIFICITY
The specificity of total T4 ELISA kit was determined by measuring interference from high concentrations of 3,5-diiodothyronine (up to 10 ug/dL), 3,3’,5-triiodothyronine (rT3,, up to 1000 ng/ml), 3,3’,5-triiodothyroacetic acid (up to 42 nmol/l), 3,3’,5- triiodothyropropionic acid (up to 63 nmol/l), Aspirin (10 mg/dL) iodoacetic acid (10 ug/dL), phenylbutazone (10 mg/dL). No significant cross-reaction was observed with any of these compounds.

6. CORRELATIVE STUDY
ADI total T4 elisa kit was compared with Inc Star Clinical assay T4 RIA by analyzing 99 samples (2.6-25.5 ug/dL). The regression analyses showed good correlation between the two assays. Inc Star RIA ADI T4 ELISA Mean Sample (ug/dL) 8.682 8.854 Slope = 0.98288; Intercept =0.790 Correlation Coefficient =0.852 ADI = 0.929 (Inc stat T4)+ 0.790

7. SPECIES REACTIVITY AND PUBLICATIONS


WORKSHEET OF TYPICAL ASSAY

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Mean A450 nm</th>
<th>A/A0 x 100 Index</th>
<th>Calculated Conc (ug d/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Std. A (0 ug/dL)</td>
<td>2.679</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>Std. B (2 ug/dL)</td>
<td>1.961</td>
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<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>Std. C (5 ug/dL)</td>
<td>1.322</td>
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<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>Std. D (10 ug/dL)</td>
<td>0.901</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>Std. E (15 ug/dL)</td>
<td>0.659</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1, F2</td>
<td>Std. F (25 ug/dL)</td>
<td>0.396</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1, G2</td>
<td>Sample 1</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT
Based on sixteen replicates determinations of the zero standard, the minimum concentration of total T4 detected using this assay is 0.5 ug/dL. The detection limit is defined as the value deviating by 2 SD from the zero standard.
2. PRECISION

Intra-assay precision:
Three serum samples (mean total T4 concentrations 3.5, 6.8, 13.9 ug/dL) were run in five separate runs. The samples showed good intra-assay precision with %CV of 9-11.

PRINCIPLE OF THE TEST

Total T4 ELISA kit is based on competitive binding of human thyroxine from serum samples and enzyme-labeled T4 to T4-specific antibodies immobilized on microtiter well plates. In the assay, total T4 is released from its binding proteins by a releasing agent present in the assay buffer. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (blue color) is inversely proportional to the amount of T4 present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. And the concentration of T4 in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Total T4 ELISA test is intended for in vitro research use only. The reagents contain proclin-300 as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses; therefore, sera should be handled with appropriate precautions. Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20oC for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION

Dilute wash buffer (1:20) with distilled water (25 ml stock in 475 ml). Store at 4oC. Enzyme conjugate (11X) dilute 1:10 with conjugation buffer (prepare 1 ml per 8-well strip or 10 ml for full plate; 1 ml stock and 10 ml buffer). Dilute conjugate in required amounts only and do not store diluted conjugate.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8oC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8oC or stored frozen in small aliquots.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute conjugate in required amounts. Dilute wash buffer (1:20) with distilled water (25 ml stock in 475 ml). Store at 4oC.
1. Label or mark the microtiter well strips to be used on the plate. Store unused strips in the sealed pouch at 4oC.
2. Pipet 25 ul of standards, control, and serum samples into appropriate wells in duplicate.
3. Add 100 ul of enzyme conjugate into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for 60 minutes at room temperature.
4. Aspirate and wash the wells 3 times with 300 ul of wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

5. Dispense 100 ul TMB substrate per well. Mix gently for 5-10 seconds.
6. Cover the plate and incubate for 15 minutes at room temperature. Blue color develops in standards and samples.
7. Stop the reaction by adding 50 ul of stop solution to all wells at the same timed intervals as in step 6. Mix gently for 5-10 seconds. Blue color turns yellow.
8. Measure the absorbance at 450 nm using an ELISA reader within 30 min.

NOTES
Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

Limitations
Serum samples containing more than 24 ug/dL T4 should be diluted with the zero standard (standard A or normal saline) and the results obtained should be multiplied by the appropriate dilution factor. Samples containing T4 <0.5 ug/dL are analyzed by diluting with 2 ug/dL T4 calibrator to extend the curve. Calibrators and controls from other manufacture should not be used as they may contain serum preservatives incompatible with ADI’s ELISA reagents. Whenever laboratory data conflicts with clinical findings or impressions, clinical judgment should be exercised and additional evaluations undertaken. Use of ADI’s reagent in a study of euthyroid patients in one geographic location will yield a normal range. It is recommended that laboratories adjust normal values to reflect geographic and population differences specific to the patients they serve.

CALCULATION OF RESULTS
Draw the standard curve on a linear graph paper by plotting net absorbance values of standards against appropriate total T4 concentrations. Read off the total T4 concentrations of the control and patient samples.

EXPECTED VALUES
In a study of 75 euthyroid patients yielded a normal range of (4-12 ug/dL) at the 95% confidence limit. It is recommended that researchers adjust normal values to reflect geographic and population differences.