Total T4 (Thyroxine) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIATT41	Total T4 ELISA	96 Tests

Intended Use:
Total T4 ELISA is intended for quantitative determination of Total Thyroxine in human serum. This reagent is for In vitro diagnostic use only.

Summary and Principle:
Measurement of T4 is an important tool for the assessment of thyroid function. Thyroxine is a major hormone produced by the thyroid gland. Thyroxine is released into the blood by a proteolytic cleavage of follicular thyroglobulin. About 0.03% of Thyroxine is unbound and it is called as Free T4. More than 99% of T4 is bound to plasma proteins. Increased levels of T4 is seen in hyperthyroidism whereas low levels of T4 is seen in congenital hypothyroidism and Hashimoto's thyroiditis.

Total T4 ELISA assay is based on the principle of competition. Microwells are coated with T4 antigen. T4 antigen in the sample competes with the microwell coated T4 antigens for binding sites on the HRP-labelled anti-T4 antibodies in the conjugate. A washing step removes unbound antigens. Addition of Substrate made of TMB and Urea peroxidase results in a chromogenic reaction with the HRP on bound antibodies which is stopped by decreasing the pH using the stop solution. The colour intensity is inversely proportional to the concentration of T4 in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with T4 antigen. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 2 months at 2-8°C.
T4 Calibrators	6x1ml	6 vials containing T4 at concentrations of 0.0, 1.0, 2.5, 5.0, 15.0 and 30.0 µg/dl made up in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable for 1 month at 2-8°C.
Enzyme Conjugate	1x6ml	HRP (horseradish peroxidase) labelled mouse monoclonal T4 in Tris-NaCl buffer containing BSA. Once open, stable for 2 months at 2-8°C.
Wash Buffer Concentrate (40X)	1x25ml	PBS-Tween at pH 7.4. 40X concentrate. Once open, stable for 2 months at 2-8°C. The concentrate must be diluted 1 to 40 with distilled water before use. Once diluted it is stable at room temperature for 2 months.
Substrate Solution	1x11ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable for 2 months at 2-8°C.
Stop Solution	1x6ml	Sulphuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

Traceability: The calibrators are manufactured using pure grade T4
Also provided: Plastic sealable bag, IFU and plate covers.

Materials required but not provided:
Distilled water, vortex mixer, micropipettes, incubator, microplate reader and microplate washer.

Specimen Collection:
Collect serum samples in accordance with correct medical practices. Cap and store the samples at 18-25°C for no more than 8 hours. Stable for 3 days at 2-8°C, and 1 month at -20°C. Recovery within 90-110% of serum value or slope 0.9-1.1. Freeze only once. Samples were tested with a selection of commercially available sample collection tubes but not all available tubes from all manufacturers have been tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test result in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide. Avoid grossly haemolytic, lipemic or turbid samples. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation should be sufficient to remove particulate matter.

Storage and Stability:
The contents of the kit are stable up to the expiry date when stored unopened at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8oC, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier. Seal and return all the other unused reagents to 2-8oC, under which conditions the stability will be retained for 2 months, or until the labelled expiry date, whichever is earlier.

Precautions and Safety:
The ELISA assay is time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify the steps.

1. **For professional use only.**
2. **Follow the instructions in this IFU as reliability of results cannot be guaranteed if there are deviations from the instructions.**
3. **The calibrators contain human serum based components. They have been tested and found to be non-reactive to HBsAg, HIV and HCV antibodies and syphilis. The assay contains materials of animal origin like BSA which have been sourced from countries where BSE has not been reported. It is recommended that all human serum based material is considered as potentially infectious and care to be taken in their use.**
4. **Wear laboratory protective equipment including gloves and safety glasses whilst handling reagents, controls and samples. Wash hands thoroughly after each operation.**
5. **Samples in the microwells should not have bubbles as these bubbles may result in erroneous results.**
6. **Wash the wells completely. Avoid overflow during wash. Remove any residual wash buffer by tapping the microwells on a clean towel or absorbent paper. Preferably use an automated microplate washer.**
7. **Use new pipette tips for each sample and reagent addition to avoid cross contamination.**
8. **Do not use kits after the expiry date.**
9. **Do not interchange components from other kits.**
10. **The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.**

Procedure:
Reagent preparation:

1. Ensure samples, calibrators, and controls are at ambient temperature (18-25°C) before measurement. Mix all reagents by gently inverting prior to use
2. Adjust the incubator to 37°C.
3. Prepare Wash Buffer from Concentrate before measurement. Stable for 2 months at ambient temperature.
4. Do not use substrate if it looks blue.
5. Do not use reagents that are contaminated or have bacterial growth.

STEP 1
Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2
Addition of Samples, Calibrators and Controls: Add 50 µl of calibrators, controls or samples to appropriate wells.

STEP 3
Addition of Enzyme Conjugate: Add 50 µl of Enzyme Conjugate solution to each well. Mix well by tapping the edge of the plate gently.

STEP 4
Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 5
Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with 350 µl of Wash Buffer. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6
Addition of the Substrate: Add 100 µl of Substrate Solution to each well.

STEP 7
Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature (18-25°C). Ensure that the incubation is done in the dark.

STEP 8
Stopping the Reaction: Add 50 µl of the Stop Solution into each well and mix gently until the well contents change from blue to yellow.

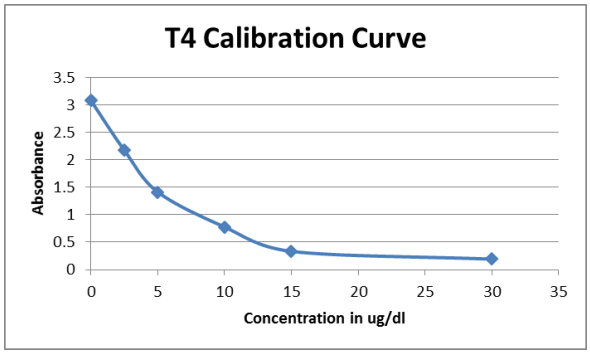
STEP 9
Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. The results should be read within 30 minutes of adding the Stop Solution. Note down the absorbances.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot Absorbance on the y axis and Concentration in µg/dl on the x axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the y axis and find the intersecting point on the curve. Read the concentration from the x axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	3.077	0.0 µg/dl
CAL B	2.172	2.5 µg/dl
CAL C	1.414	5.0 µg/dl
CAL D	0.777	10.0 µg/dl
CAL E	0.334	15.0 µg/dl
CAL F	0.191	30.0 µg/dl
Control Level 1	0.943	8.7 µg/dl
Control Level 2	0.386	14.41 µg/dl
Sample	0.863	9.32 µg/dl



Prestige Diagnostics UK Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

Limitations:

• The assay is unaffected by icterus (bilirubin < 600 µmol/l or < 35 mg/dl), haemolysis (Hb < 0.559 mmol/l or < 0.9 g/dl), lipemia (Intralipid < 1200 mg/dl), and biotin < 94 nmol/L or < 23 ng/ml. Criterion: Recovery within ± 10 % of initial value.
• Heterophilic antibodies and rheumatoid factors in samples may interfere with test results by reacting with reagent immunoglobulins. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. Samples from patients on therapies involving animal derived products are not suitable to be tested by this assay.
• Performance of this test has not been established with neonatal samples.
• Serum T4 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, TBG concentration, and the binding of T4 to TBG. Thus, total T3 concentration alone is not sufficient to assess clinical status.
• Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
• The Total T4 result from this test should not be used as the sole criteria for the diagnosis of thyroid status, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

Calculation:

Where calibrator details are entered into the microplate reader, the analyser automatically calculates the analyte concentration of each sample.
Conversion factors:
nmol/l x 0.0777 = µg/dl
µg/dl x 12.872 = nmol/l
nmol/l x 0.777 = µg/l

Performance Characteristics:

Measuring range

0.5 - 30 µg/dl or 6.43 - 386 nmol/l (defined by the lower limit of detection and the maximum of the master curve). Values below the detection limit are reported as < 0.5 µg/dl or 6.43 nmol/l. Values above the measuring range are reported as >25.0 µg/dl or 385.80 nmol/l.

Lower limit of measurement

Lower limit of detection: 0.5 µg/dl (6.43 nmol/l).
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value equal to the mean plus two x standard deviations of the concentration of the zero standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Expected values

Measurement of 2046 serum samples from euthyroid test subjects using the Total T4 ELISA kit yielded the following values (2.5th-97.5th percentile):
4.5 - 13.8 µg/dl or 57.9 - 177.6 nmol/l
The test panel did not include samples from children, adolescents or pregnant women so the reference range may not be applicable to these groups.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Precision

Precision was determined using reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n= 40). The following results were obtained for Intra Assay precision:

Panel	Mean	SD	CV%
Serum pool 1	3.94	0.36	9.12
Serum pool 2	9.27	0.51	5.48
Serum pool 3	15.84	0.72	4.57
Control Level 1	7.33	0.32	4.36
Control Level 2	15.64	0.57	3.66

Method comparison

A comparison of the Total T4 ELISA (y) with another commercial T4 ELISA (x) using clinical samples (sample concentration range was approximately 1 to 25 µg/dl) gave the following Linear Regression (n= 91):

y = 1.0296 x - 1.2318, r = 0.9689

Analytical specificity

For the antibody derivative used, the following cross-reactivities were found: L-T4 and D-T4 100 %; L-T3 1.89 %; D-T3 1.44 %; 3-iodo-L-tyrosine 0.002 %; 3,5-diiodo-L-tyrosine 0.008 %.

Quality Control:

It is recommended that each test run should include quality controls at low, normal and elevated levels, the concentration results of which should fall within the assigned range for the analyte.

References:

Wheeler MH, Lazarus JH. Diseases of the Thyroid. London,Glasgow, Weinheim, New York, Tokyo, Melbourne, Madras: Chapman and Hall Medical, 1994:108-115.
2. Pfannenstiel P, Saller B. Schilddrüsenkrankheiten Diagnose und Therapie. Berliner Medizinische Verlagsanstalt GmbH, 1995;2:43-62,97-106.
3. Wenzel KW. Pharmacological interference with in vitro tests of thyroid function. Metabolism 1981;30(7):717-732.
4. Burrow GN. Thyroid status in normal pregnancy. J Clin Endocrinol Metab 1990;71:274-275.
5. Lazarus JH, Othman S. Thyroid disease in relation to pregnancy. Clin Endocrinol 1991;34:91-98.
6. Fisher DA. Physiological variations in thyroid hormones; physiological and pathophysiological considerations. Clinical Chemistry 1996;42:1,135-139.
7. Burrow GN, Fisher DA, Larsen PR. Maternal and faetal thyroid function. N Eng J Med 1994;331:1072-1078.
8. Nelson JC, Wilcox RB. Analytical performance of free and total thyroxine assays. Clinical Chemistry 1996;42:1,146-154.

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by Date
	Manufacturer		

CE

Prestige Diagnostics UK Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

V5: rev Feb 2017