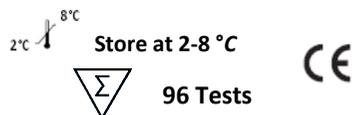


T4 ELISA

Microplate based ELISA (enzyme linked immunosorbent assay) for the quantitative determination of T4 (thyroxine) in human serum

IVD For *in vitro* diagnostic and professional use only



Intended use

Immunoassay for the *in vitro* quantitative determination of thyroxine in human serum.

Introduction

The hormone thyroxine (T4) is the main product secreted by the thyroid gland and is an integral component of the hypothalamus anterior pituitary-thyroid regulating system. It has the function of anabolically influencing metabolism. Thyroxine is formed in a coupling reaction from two DIT molecules (3,5-diiodotyrosine) in the thyroid gland. It is stored bound to thyroglobulin in the Lumina of the thyroid follicles and is secreted as required under the influence of TSH.

The major part (> 99 %) of total thyroxine (T4) in serum is present in protein-bound form. As the concentrations of the transport proteins in serum are subject to exogenous and endogenous effects, the status of the binding proteins must also be taken into account in the assessment of the thyroid hormone concentration in serum. If this is ignored, changes in the binding proteins (e.g. due to estrogen-containing preparations, during pregnancy or in the presence of a nephritic syndrome etc.) can lead to erroneous assessments of the thyroid metabolic state.

The determination of T4 can be utilized for the following indications: the detection of hyperthyroidism, the detection of primary and secondary hypothyroidism, and the monitoring of TSH-suppression therapy.

The ATLAS ENZYCODE T4 assay employs a competitive test principle with an antibody specifically directed against T4. Endogenous T4, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS).

Test Principle

Competition principle. Total duration of assay: **80 minutes**.

- Sample, T4 derivant coated microwells and enzyme labeled Anti-T4 are combined.
- During the incubation, T4 derivant coated on microwells and T4 present in the sample compete for binding to the enzyme labeled antibodies.
- After washing, a complex is generated between the solid phase and enzyme linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The color intensity is inversely proportional to the amount of T4 in the sample.

Materials

Materials provided

1. **Coated Microplate:** 8 x 12 strips, 96 wells, pre-coated with T4 derivant.
2. **Calibrators:** Six white cap vials, 1 ml each, ready to use; Concentrations: 0(A), 2.5(B), 5(C), 10(D), 15(E) and 30(F) µg/dL.
3. **Enzyme Conjugate:** One red cap vial, 6.0 ml of HRP (horseradish peroxidase) labeled mouse monoclonal T4 in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.2% ProClin300® preservative. ANS 1.5 mg/mL.
4. **Substrate:** One brown cap vial, 11ml, ready to use, (tetra methyl benzidine) TMB.
5. **Stop Solution:** One yellow cap vial, 6.0 ml of 1 mol/l sulfuric acid.
6. **Wash Solution Concentrate (40X):** One transparent cap bottle, 25 ml, PBS-Tween wash solution.
7. **IFU,** 1 copy.
8. **Plate Lid:** 1 piece.

Materials required (but not provided)

1. Microplate reader with 450nm and 620nm wavelength absorbent capability.
2. Microplate washer.
3. Incubator.
4. Plate shaker.
5. Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
6. Absorbent paper.
7. Distilled water.

Warnings and Precautions

1. For *in vitro* diagnostic use only. For professional use only.
2. All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II. But all

products should be reared as potential biohazards in use and for disposal.

3. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
4. Conduct the assay away from bad ambient conditions. E.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and soon, or containing dust.
5. Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Do not use reagents beyond the labeled expiry date.
8. Do not mix or use components from kits with different batch codes.
9. If more than one plate is used, it is recommended to repeat the calibration curve.
10. It is important that the time of reaction in each well is held constant to achieve reproducible results.
11. Ensure that the bottom of the plate is clean and dry.
12. Ensure that no bubbles are present on the surface of the liquid before reading the plate.
13. The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

Storage

1. Store at 2-8°C.
2. Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
3. Seal and return unused calibrators to 2-8 °C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles.
4. Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation

1. Collect serum samples in accordance with correct medical practices.
2. 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.

3. The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results 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.
4. Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
5. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
8. 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 conditions should be sufficient to remove particulate matter.
9. Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
10. Adjust the incubator to 37 °C.
11. Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature
12. Don't use Substrate if it looks blue.
13. Don't use reagents that are contaminated or have bacteria growth.

Quality control

Each laboratory should assay controls at levels in the low, normal, and elevated range for monitoring assay performance. There controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirements for this assay are to purchase trueness control materials separately and test them together with the samples

within the same run. The result is valid if the control values fall within the concentration range sprinted on the labels.

Wash solution (40X dilution)

Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Procedure

1. Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
2. Add **50 µL of calibrators or samples** to each well.
3. Add **50 µL of enzyme conjugate** to each well.
4. Shake the microplate gently for 30 seconds to mix.
5. Cover the plate with a plate lid and incubate at 37 °C for 60 minutes.
6. Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add **350 µl of wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
8. Add **100 µl of substrate** to each well.
9. Incubate at ambient temperature (18-25°C) in the dark for reaction for 20 minutes. Do not shake the plate after substrate addition.
10. Add **50 µl of stop solution** to each well.
11. Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
12. Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

Calculation

1. Record the absorbance obtained from the printout of the microplate reader.
2. Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
3. Plot the common logarithm of absorbance against concentration in µg/dl for each calibrator.
4. Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (µg/dL)	Absorbance
Cal A	0	3.077
Cal B	2.5	2.172
Cal C	5	1.414
Cal D	10	0.777
Cal E	15	0.334
Cal F	30	0.191
Control 1	8.7	0.943
Control 2	14.41	0.386
Sample	9.32	0.863

Limitations - interference

1. The assay is unaffected by Ictinus (bilirubin < 600 µmol/L or <35 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.9 g/dL), lipemia (Intralipid < 1200 mg/dL), and biotin < 94 nmol/L or <23 ng/mL.
2. Criterion: Recovery within ± 10 % of initial value.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. 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. This kind of samples is not suitable to be tested by this assay.
4. Performance of this test has not been established with neonatal samples.
5. 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.
6. 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.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, µg/dL or µg/L).

Conversion factors:

$$\text{nmol/L} \times 0.077688 = \mu\text{g/dL}$$

$$\mu\text{g/dL} \times 12.872 = \text{nmol/L}$$

nmol/L x 0.77688 = µg/L

Limits and ranges

Measuring range

0.5-30.0 µg/dL or 6.43-385.80 nmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.5 µg/dL or 6.43nmol/L. Values above the measuring range are reported as >30.0 µg/dL or 385.80 nmol/L.

Lower limits of measurement

Lower detection limit

Lower detection limit: 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 lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Expected values

Measurement with the ATLAS T4 assay on 2046 serum samples from euthyroid test subjects in China yielded the following values (2.5th-97.5th percentile):

4.5-13.8µg/dL or 57.9-177.6 nmol/L.

We have not studied the reference intervals in children, adolescents and pregnant women. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using ATLAS 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:

Sample	Mean µg/dL	Repeatability*		Intermediate precision	
		SD ng/mL	CV%	SD µg/dL	CV%
Human Serum 1	3.94	0.36	9.14	0.35	8.88
Human Serum 2	9.27	0.51	5.50	0.68	7.32
Human Serum 3	15.84	0.72	4.55	0.79	4.99
PC	7.33	0.32	4.36	0.27	3.68

Universal 1					
PC	15.64	0.57	3.64	0.85	5.43
Universal 2					

*Repeatability = within-run precision

Method comparison

A comparison of the ATLAS T4 assay (y) with the Roche Cobas T4 (x) using clinical samples gave the following correlations: Number of samples measured: 91

Linear regression

y = 1.0296x - 1.2318

r = 0.9689

The sample concentrations were between approx. 1 and 25 µg/dL.

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 %.

References

1. 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. Schilddrüsenerkrankungen 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 fetal thyroid function. N Engl 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.

 **ATLAS Medical GmbH**
Ludwig-Erhard Ring 3
15827 Blankenfelde-Mahlow
Germany
Tel: +49 - 33708 – 3550 30
Email: Info@atlas-medical.com
Website: www.atlas-medical.com

PPI1448A01

Rev C (12.04.2022)

	Catalogue Number		Temperature limit
	In Vitro diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry