FREE T4 ELISA

IVD For *in vitro* diagnostic and professional use only

2°C 8°C **Store at 2 to 8°C**

CE

Σ **96 Tests**

INTENDED USE

Atlas Free T4 Kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of free thyroxine (FT4) in human serum. This assay is used to diagnosis, monitoring and treatment of thyroid disorders.

INTRODUCTION

Thyroid hormone thyroxine (T4) is physiologically part of the regulating circuit of the thyroid gland and has an effect on general metabolism. The major fraction of the total thyroxine is bound to transport proteins (TBG, prealbumin, and albumin). The free thyroxine (fT4) is the physiologically active thyroxine component. The determination of free thyroxine is an important element in clinical routine diagnostics. Free T4 is measured together with TSH when thyroid function disorders are suspected. The determination of FT4 is also suitable for monitoring thyrosuppressive therapy. The determination of free T4 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary.

PRINCIPLE OF THE TEST

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 FT4 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 FT4 in the sample.

KIT COMPONENTS

Materials provided

- **Coated Microplate**, 8 x 12 strips, 96 wells, pre-coated with T4 derivant.
- **Calibrators**, 6 White cap vials, 1 ml each, ready to use; Concentrations: 0 (A), 5 (B), 10 (C), 20 (D), 50 (E) and 100 (F) pmol/L.
- **Enzyme Conjugate**, 1 Red cap vial, 6 ml of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-T4 in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.2% ProClin300® preservative.
- **Substrate**, 1 Brown cap vial, 11ml, (tetramethylbenzidine) TMB.
- **Stop Solution**, 1 Yellow cap vial, 6.0 ml of 1 mol/l sulfuric acid.
- **Wash Solution Concentrate,** 1 Transparent bottle, 25 ml (40X concentrated), PBS-Tween wash solution.
- **One copy of package insert.**
- **One piece of plate cover.**

Materials required but not provided

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

Packaging Contents

REF **8.12.03.0.0096**

Contains reagents enough to make 96 tests.

STORAGE

- Store at 2-8°C.
- 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.
- 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.

PRECAUTIONS AND WARNINGS

- For *in vitro* diagnostic and professional use only.
- 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.
- Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
- 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.
- 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.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- If more than one plate is used, it is recommended to repeat the calibration curve.
- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Ensure that the bottom of the plate is clean and dry.
- Ensure that no bubbles are present on the surface of the liquid before reading the plate.
- The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.
- **Any suspected serious incidents related to this assay shall be immediately reported to Atlas Medical Authorized Representative in the EU, and the national competent authorities of the Member States where the users and/or patients are located.**

COLLECTION, HANDLING AND PREPARATION OF SPECIMEN

- Collect serum specimens in accordance with correct medical practices.
- Cap and store the specimens at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8 °C, and 1 month at -20 °C. **Freeze only once.**
- Do not use heat-inactivated specimens.
- 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.
- Avoid grossly hemolytic, lipemic or turbid samples.
- 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 requirement 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 ranges printed on the labels.

TEST PROCEDURE

- Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
- Add 50 µL of calibrators or samples to each well.
- Add 50 µL of enzyme conjugate to each well.
- Shake the microplate gently for 30 seconds to mix.
- Cover the plate with a plate lid and incubate at 37 °C for 60 minutes.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- 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.
- Add 100 µL of substrate to each well.
- Incubate at ambient temperature (18-25°C） in the dark for reaction for 20 minutes. Do not shake the plate after substrate addition.
- Add 50 µL of stop solution to each well.
- 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.
- 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

- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in pmol/L for each calibrator.
- 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

LIMITATIONS

Sample	Value (pmol/L)	Absorbance
Calibrator A	0	3.084
Calibrator B	5	2.121
Calibrator C	10	1.842
Calibrator D	20	0.915
Calibrator E	50	0.557
Calibrator F	100	0.253
Control 1	10.29	1.824
Control 2	33.38	0.795
Sample	17.02	1.408

- The assay is unaffected by icterus (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.
- Criterion: Recovery within ± 10 % of initial value.
- 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.
- Performance of this test has not been established with neonatal samples.
- In NTI (severe nonthyroidal illness), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction.
- Serum FT4 values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
- The interpretation of FT4 is complicated by a variety of drugs that can affect the binding of T4 to the thyroid hormone carrier proteins or interfere with its metabolism to T3.
- In rare conditions associated with extreme variations in albumin binding capacity for T4-such as FDH (familial dysalbuminemic hyperthyroxinemia)-direct assessment of FT4 may be misleading.
- Circulating antibodies to T4 and hormone binding inhibitors may interfere with the performance of the assay.
- If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 100 pmol/l). Do not try to dilute the samples. TBG variations in different matrices will not allow FT4 hormone to dilute serially.
- A decrease in FT4 values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect FT4 values, has been compiled by the Journal of the American Association of Clinical Chemists.
- Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Antimouse 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 pmol/L, ng/dL or ng/L).

Conversion factors:

pmol/L x 0.077688 = ng/dL

ng/dL x 12.872 = pmol/L

pmol/L x 0.77688 = ng/L

LIMITS AND RANGES

Measuring range

2-100 pmol/L or 0.155-7.77 ng/dL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 2 pmol/L or 0.155 ng/dL. Values above the measuring range are reported as > 100 pmol/L or 7.77 ng/dL.

Lower limits of measurement

Lower detection limit

2 pmol/L or 0.155 ng/dL

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

11.5-23.8 pmol/L or 0.893-1.849 ng/dL

These values correspond to the 2.5^{th} and 97.5^{th} percentiles of results obtained from a total of 777 healthy test subjects examined.

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.

PERFORMANCE CHARACTERISTICS

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:

		Repeatability*		Intermediate precision	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human Serum 1	14.36	1.10	7.66	1.25	8.72
Human Serum 2	28.51	1.95	6.84	2.13	7.46
Human Serum 3	45.44	2.96	6.51	3.32	7.31
PC Universal 1	17.33	1.28	7.39	1.19	6.88
PC Universal 2	32.15	2.31	7.19	2.23	6.93

*Repeatability = within-run precision

Method comparison

A comparison of the Atlas FT4 assay (y) with the Roche Cobas FT4 (x) using clinical samples gave the following correlations: Number of samples measured: 91

Linear regression

y = 1.0595x - 1.5237

r = 0.9695

The sample concentrations were between approx. 4 and 100 pmol/L.

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 %.

Functional Sensitivity

2.15 pmol/L or 0.167ng/dL

Functional sensitivity is lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20%.

REFERENCES

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	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