



T4 ELISA KIT

Enzyme Immunoassay for Quantitative Determination of Thyroxin (T4) in Human Serum

1. INTENDED USE

This kit is intended for the quantitative determination of thyroxin- T4 in human serum.

Thyroxin is one of the thyroid gland hormones with a molecular weight of 777 Da. Quantitative determination of serum thyroxin is significant for evaluation of thyroid gland function.

2. ASSAY PRENCIPLE

"thyroidEIA-thyroxin-01" is a solid phase immunoassay, based on competition between horseradish peroxidase-labeled thyroxin and thyroxin of serum sample for monoclonal anti-thyroxin antibodies, immobilized on a plastic surface of microwells. Calibrators, control and serum samples containing thyroxin inhibit the binding of the enzyme -labeled thyroxin to immobilized antibody molecules. After incubation on shaker, enzyme-labeled thyroxin not bound to the solid phase is removed by repeated washing. Then TMB substrate solution is added and the coloring appears. The intensity of appearing color is inversely proportional to the thyroxin concentration in serum samples. The enzyme reaction is stopped by dispensing the acidic solution (1N HCL) into the wells. Optical density of the solution in the wells is inversely proportional to the T4 concentration in the calibrators (x-axis) and their corresponding OD values (y-axis). The T4 concentration of the specimen is directly read off from the standard curve.

2. KIT CONTENTS

- 12 microtiterplate, 12x8 wells, coated with anti-T4 monoclonal antibodies.
- T4 calibrators (serum-based solution containing known T4 concentrations).
- conjugate contains thyroxin with HRP.
- concentrated wash buffer.
- TMB substrate, tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide .
- "Stop reagent".
- control (serum-based buffer containing known T4 concentration), see vial label.
- ANSA-T4 solution: buffered solution of 8-anilino-1-naphtalenesuphonic acid.

PATIENT'S SAMPLE

Specimen collection and storage:

Blood is taken aseptically by venipuncture. After clotting, the serum is separated by centrifugation. Do not use plasma, hemolysed or lipemic serum and serum with sodium azide added as preservative.

Store serum samples at 2-8 for no more than 2 days; for longer storage it is recommended to aliquote and freeze at -20 or below. Avoid repeated freezing.

Preparation before use:

Prior to assay, allow the samples to reach room temperature. Take care to agitate serum samples gently in order to ensure homogeneity.

DATA PROCESSING

Data processing is done by a computer-assisted analysis by plotting the mean OD values of the calibrators at 450 nm versus their respective T4 concentrations using 4PL or 5PL fit (see typical standard curve) . Mean OD of the A1 –A2 wells is used as blank.

Any extrapolation of the standard curve to T4 concentration above the nominal value of Calibrator 5 (approximately 400 U/ml) is not permitted.

REFERENCE VALUES

Serum samples collected between 9 and 11 a.m. from 40 apparently healthy people (both males and females) at the age of 21-45, were assayed with ThyroidEIA-thyroxin-01 kit. Thyroxin concentrations range was 53-158 nmol/l mean 98 nmol/l) these limits should be considered as guidelines only.

It is highly recommended for each laboratory to determine its own reference range of T4 concentrations.

3. PERFORMANCE CHARACTERISTICS

Dilution parallelism of serum samples:

Serial dilutions of three human serum samples with predetermined cortisol concentration in calibrator 0 (0 nmol/l) were assayed with thyroidEIA-thyroxin-01 kit with the following:

sample	dilution	Measured concentration, nmol/l	Expected concentration, nmol/l	Observed/expected concentration ratio, %
1	Undiluted	72		
	1:2	36	34	94%
	1:4	18	17	94%
	1:8	9.0	9.2	102%
2	Undiluted	126		
	1:2	63	64	102%
	1:4	32	31	97%
	1:8	16	15	94%
	1:16	8.0	7.6	95%
3	Undiluted	145		

	1:2	73	79	108%
	1:4	36	35	97%
	1:8	18	16	89%
	1:16	9.0	9.4	104%

Specificity:

Cross reaction of anti- thyroxin monoclonal antibodies with different thyroids is shown in the table:

thyroid	Cross-reaction, %
L-Thyroxin	100
L-Triiodothyronine	8
L-Diiodotyrosine	0.001

Analytical sensitivity (lower detection limit):

Analytical sensitivity of thyroidEIA-thyroxine-01 assay, or the lowest detectable concentration that can be distinguished from zero calibrator, is 5 nmol/l. it is defined as mean OD of 10 replicates of calibrator 0 minus 2 standard deviations.

5. MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT:

- digital variable pipettes that cover volume range from 0.005 to 5ml , with appropriate disposable tips;
- 8-channel digital variable pipette that covers volume range up to 0.3 ml, with appropriate disposable tips;
- microplate shaker-thermostat , able to maintain temperature +37°C and shaking speed 500 to 800 rpm;
- automatic microplate reader;
- volumetric cylinder;
- volumetric beaker;
- distilled water.

SIZE AND STORAGE

ThyroidEIA-thyroxine-01 kit is designed for 96 determination. This is sufficient for 40 unknowns, 6 calibrators, 1 control serum and 1 TMB substrate control in duplicates, provided that all the strips are used simultaneously.

Please take into consideration that calibrators should be measured in each separate assay.

It is also recommended to measure T4 concentration in the control each time

NOTE: if used partially, kit should be utilized within a month after opening.

The expiry date of the kit is reported on the box label, expiry date for each component is indicated on the respective label.

Upon receipt, ThyroidEIA-thyroxine-01 kit should be stored at 2-8°C, preferably in the original kit box.

If used for separate experiments, kit content should be stored as follows:

- the unused strips: in a firmly closed plastic bag at 2-8°C until expiry date.
- opened vials with conjugate and TMB substrate solution at 2-8°C for no more than 1 month.
- concentrated wash buffer : at 2-8°C until expiry date.
- wash buffer prepared for use, in a firmly closed bottle for no more than 5 days at room temperature.
- reconstituted calibrators and control: at 2-8°C for no more than 1 month after reconstitution.
- ready to use calibrators and control: at 2-8°C for no more than 1 month after opening.
- stop reagent at 2-8°C until expiry date.
- ANSA-T4 solution: at 2-8 until expiry date, protected from light.

6. REAGENT PREPARATION FOR ASSAY

Before the assay, allow all the kit components to reach room temperature and stir thoroughly.

6.1. Microtitration strips. Before opening keep the bag at room temperature (+18...25°C) for 30 minutes. Open the bag and place required number of strips on strip holder. Put remaining strips back in plastic bag and close tightly. Keep at +2...8°C until expiry date stated on the label.

6.2. Wash buffer. Prepare the necessary volume of Wash buffer by dilution of Buffer 20-fold with distilled water. For example:

5 ml of Buffer + 95 ml of distilled water. Mix thoroughly, avoiding foaming.

Keep firmly closed. Store at room temperature (+18...25°C) for no more than 5 days. The rest of the Buffer should be stored firmly closed at +2...8°C until expiry date.

6.3. calibrators and control:

Lyophilized calibrators and control: gently tap on the vial caps to shake off all the dry matter. Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5 ml of distilled water to vials with lyophilized calibrators and control, close each vial with the corresponding cap and leave for 10 minutes at room temperature 18-25°C, then stir gently, avoid foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at room temperature with periodical gentle stirring. Make sure that no dry matter is left on the caps and walls of the vials. Store at 2-8°C for no more than 1 month.

Liquid calibrators and control are ready for use. Once opened, store at 2-8 for no more than 1 month.

6.4. protect the ANSA-T4 solution from direct light.

6.5. protect the TMB solution from direct light.

7. ASSAY PROCEDURE

All the samples should be tested in duplicates

7.1. All the components and serum samples should be brought to room temperature and stirred thoroughly before the assay.

7.2. dispense 100µl of ANSA-T4 solution into each well, except A1-A2 wells.

7.3. dispense

20 µl of T4 calibrators(0-5)

20 µl of T4 control serum.

20 µl of patient samples, into the respective wells.

Note: total time of dispensing must not exceed 15 minutes , otherwise the test result may be unreliable, because the time of incubation with conjugate will substantially vary for different samples.

7.5. Pipette 100 µl of conjugate into each well **except A1 and A2.**

7.6. Incubate strips for 1 hour while shaking at +37°C.

7.7. decant, then wash each well 4 times with wash buffer. Each time add 300 µl of wash buffer per well and shake for 5–10 sec, then aspirate the buffer. After the last washing cycle, if necessary, invert the plate and firmly tap on a clean paper towel to remove remaining wash buffer.

7.8. Immediately Add 100 µl of TMB Substrate solution into each well. Incubate at room temperature (+18...25°C) in the dark for 15-30 minutes, depending on the color intensity.

7.9. Add 100 µl of Stop reagent to all wells and shake well for 1-2 minutes.

7.10. Read the optical density of the solution in the microwells at a wavelength of 450 nm.

SAFETY PRECAUTIONS

- this kit is for in vitro diagnostic use only. The operator should be thoroughly follow the manual to obtain the reliable data. This instruction manual is valid only for the present kit with the listed composition. Any exchange of kit components is not allowed by CE regulations.

- do not use kits or components after expiry date stated on the label. take into consideration stability period for reconstituted reagents.

- do not mix or use together reagents of different lots.

- stop reagent is 1N HCL solution. Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water and seek medical advice.

- source materials of human origin that were used in preparation of kit were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. However, no known laboratory test guarantees the absence of these viral agents. Therefore, all the kit components and patient's samples should be handled as potentially hazardous.

- As the kit contains potentially hazardous material, the following precautions should be observed:

*Do not smoke, eat or drink while performing the assay.

- *Always use protective gloves.
- *Never pipette material by mouth.
- *In the case of spilling, wipe up the spills promptly and wash the affected area thoroughly with decontaminant.
- GLP and all general and individual regulations should be applied to the use of the kit.

ATLAS MEDICAL
William James House, Cowley Road, Cambridge
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524
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