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Instructions for use

T4 ELISA 2nd Generation

REF TF E-2400R

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

1. INTRODUCTION

1.1 Intended Use

The **T4 ELISA** is an enzyme immunoassay for the quantitative measurement of total Thyroxin (T4) in serum or plasma (EDTA, lithium heparin or citrate plasma).

2. PRINCIPLE OF THE TEST

The T4 Total ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the **principle of competitive binding**.
The microtiter wells are coated with a monoclonal [mouse] antibody directed towards an antigenic site of the T4 molecule.
Endogenous T4 of a sample competes with a T4-horseradish peroxidase conjugate for binding to the coated antibody.
After a washing step, to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is abruptly stopped by addition of stop solution and optical density (OD) of the resulting yellow product is measured. The intensity of colour is inversely proportional to the concentration of the analyte in the sample.
A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3. WARNINGS AND PRECAUTIONS

1. This kit is for research use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the optical density readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4. REAGENTS

4.1 Reagents provided

TF E-2431 96 **Microtiterwells**

Content: 12 x 8 (break apart) strips, 96 wells;
Wells coated with anti-T4 antibody (monoclonal)

Standards and **Controls -** ready to use

Cat. no.	Symbol	Standard / Control	Concentration	Volume/Vial
TF E-2401	STANDARD A	**Standard A**	0.0 nmol/l	0.5 ml
TF E-2402	STANDARD B	**Standard B**	25 nmol/l	0.5 ml
TF E-2403	STANDARD C	**Standard C**	50 nmol/l	0.5 ml
TF E-2404	STANDARD D	**Standard D**	100 nmol/l	0.5 ml
TF E-2405	STANDARD E	**Standard E**	175 nmol/l	0.5 ml
TF E-2406	STANDARD F	**Standard F**	250 nmol/l	0.5 ml
TF E-2451	CONTROL 1	**Control 1**	For control values and ranges please refer to vial label or QC-Datasheet.	0.5 ml
TF E-2452	CONTROL 2	**Control 2**		0.5 ml

Contents: The standards are calibrated against the following reference material: Certified Reference Material IRMM-468
Contain non-mercury preservative.

Conversion: 1 nmol/l = 0.776 ng/ml

TF E-2440 CONJUGATE **Enzyme Conjugate** - ready to use

Contents: T4 conjugated to horseradish peroxidase;
Contains non-mercury preservative.

Volume: 1 x 12 ml

TF E-0055 SUBSTRATE **Substrate Solution** - ready to use

Contents: Tetramethylbenzidine (TMB).

Volume: 1 x 12 ml

FR E-0080 STOP-SOLN **Stop Solution**- ready to use

Contents: contains 0.5 M H_2SO_4;
Avoid contact with the stop solution. It may cause skin irritations and burns.

Volume: 1 x 14 ml

Hazard identification:

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

FR E-0030 WASH- CONC 40x **Wash Solution**- 40X concentrated

Volume: 1 x 30 ml

See "Reagent Preparation".

Note: Additional *Standard A* for sample dilution is available upon request.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Wash Solution
Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 ml of concentrated *Wash Solution* with 1170 ml distilled water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, the manufactuer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5. SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA, lithium heparin or citrate plasma) can be used in this assay.

Do not use haemolytic, icteric or lipaemic specimens.
Note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Donors receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.
Specimens stored for a longer time (up to 8 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard A* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:
a) dilution 1:10: 10 µl sample + 90 µl *Standard A* (mix thoroughly)
b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl *Standard A* (mix thoroughly).

6. ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **10 µl** of each ***Standard, Control*** and **sample** with new disposable tips into appropriate wells.
3.	Incubate for **5 minutes** at room temperature (18 °C - 25 °C).
4.	Dispense **100 µl** ***Enzyme Conjugate*** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5.	Incubate for **80 minutes** at room temperature (18 °C - 25 °C).
6.	Rinse the wells **5 times** with **400 µl** diluted *Wash Solution* per well, if a plate washer is used. - OR - Briskly shake out the contents of the wells. Rinse the wells **5 times** with **300 µl** diluted *Wash Solution* per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets. **Important note:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7.	Add **100 µl** of ***Substrate Solution*** to each well.
8.	Incubate for **10 minutes** at room temperature **(18 °C - 25 °C)** **- or -** Incubate for **7 minutes** at room temperature **(26 °C - 29 °C)** **- or -** Incubate for **5 minutes** at room temperature **(more than 29 °C)**
9.	Stop the enzymatic reaction by adding **100 µl** of ***Stop Solution*** to each well.
10.	Determine the optical density of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)**. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average optical density (OD) values for each set of standards, controls and samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4-Parameter Rodbard or 4-Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 250 nmol/l. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

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

Standard	Optical Density (450 nm)
Standard A (0 nmol/l)	2.05
Standard B (25 nmol/l)	1.43
Standard C (50 nmol/l)	0.95
Standard D (100 nmol/l)	0.53
Standard E (175 nmol/l)	0.32
Standard F (250 nmol/l)	0.20

7. EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with euthyroid adult population, using the T4 ELISA the following values are observed:

Population	n	Mean (nmol/l)	Median (nmol/l)	2.5th - 97.5th Percentile (nmol/l)	Range (min. - max.) (nmol/l)
Adults	115	87.2	82.1	56.7 – 143.7	51.2 – 159.3

Total serum thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG (3, 4).

8. QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials donor results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

9. PERFORMANCE CHRACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 8.0 nmol/l – 250 nmol/l.

9.2 Specificity of Antibodies (Cross Reactivity)

The following table shows the % cross-reaction of the antibody as determined by the manufacturer.

Compound	% Cross-reactivity
T3 (3,3`,5-triiodothyronine)	1.5%
rT3 (3,3`,5-triiodothyronine, reverse T3)	1.5%
3,5-Diiodothyronine	< 0.1%

The following table shows the result of cross-reactivity tested with the T4 ELISA

Compound	concentration	Result in nmol T4/l	% Cross-reactivity
T3 (3,3`,5-triiodothyronine)	10 ng/ml	< 8 nmol/l	Not detected
Acetylsalecyl Acid	1000 µg/ml	< 8 nmol/l	Not detected
Salicylin Acid	1000 µg/ml	< 8 nmol/l	Not detected

9.3 Sensitivity

The analytical sensitivity of the ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard 0 (S0) and was found to be 8.0 nmol/l.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (nmol/l)	CV (%)
1	20	66.7	5.2
2	20	91.6	3.6
3	20	133.2	3.6

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (nmol/l)	CV (%)
1	10	73.4	4.9
2	10	136.3	5.4
3	10	200.9	8.1

9.5 Recovery

Samples have been spiked by adding T4 solutions with known concentrations in a 1:1 ratio.
The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous T4 + added T4) / 2; because of a 1:2 dilution of serum with spike material).

		Sample 1	Sample 2	Sample 3
Concentration [nmol/l]		42.8	79.7	115.0
Average Recovery [%]		99.3	102.0	105.5
Range of Recovery [%]	from	97.0	96.5	103.9
	to	101.6	106.6	107.3

9.6 Linearity

		Sample 1	Sample 2	Sample 3
Concentration [nmol/l]		122.3	129.9	142.6
Average Recovery [%]		105.7	107.0	97.5
Range of Recovery [%]	from	102.1	102.3	92.5
	to	108.4	113.7	107.0

10. LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 Drug Interferences

Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
A decrease in total thyroxine values is found with administration of testosterone, diphenylhydantoin or salicylates.

10.2 High-Dose-Hook Effect

A High-Dose-Hook Effect is not known for competitive assays.

11. REFERENCES / LITERATURE

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!