Instructions for Use

Free Thyroxine (fT4) ELISA

Enzyme immunoassay for the direct quantitative determination of free Thyroxine (fT4) in human serum or plasma.

REF **RE55241**

Σ **96**

2-8 °C

EU: IVD CE

U.S.: *For research use only.*
Not for use in diagnostic procedures.

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1. INTENDED USE

Enzyme immunoassay for the direct quantitative determination of FT4 (free Thyroxine) in human serum or plasma.

2. SUMMARY AND EXPLANATION

The thyroid hormone, thyroxine (T4) is produced by the thyroid gland. An important component in the synthesis is iodine. The major form of thyroid hormone in the blood is thyroxine (T4). Thyroxine is converted to the active T3 (three to four times more potent than T4) within cells by deiodinases (5'- iodinase). Thyroxine-binding globulin (TGB) is the major carrier protein for circulating thyroid hormone. Only a very small fraction of the circulating hormone is free (unbound) - T4 0.03%. When thyroid hormone is bound, it is not active, so the amount of FT3/FT4 is what is important. For this reason, measuring total thyroxine in the blood can be misleading. The concentration of free thyroid hormones in the blood is regulated by a negative feedback mechanism involving TSH. The binding of T4 by TBG plays a key role in this feedback mechanism and the most significant changes that occur in T4 binding capacity are the result of alterations in TBG. Changes in the circulating levels of TBG will result in a proportional increase or decrease in the concentration of total T4. However, measurement of serum FT4 is unaffected by changes in T4 protein binding levels and therefore correlates well with the functional thyroid state in most individuals. Factors responsible for discrepancies between serum total T4 levels and true thyroid states include TBG concentration, estrogenic hormones (pregnancy, oral contraceptives and estrogen) and drugs that bind to TBG preventing its binding to FT4 The thyronines act on the body to increase the basal metabolic rate, affect protein synthesis and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. Numerous physiological and pathological stimuli influence thyroid hormone synthesis. Thyrotoxicosis or hyperthyroidism is the clinical syndrome caused by an excess of circulating free thyroxine, free triiodothyronine, or both. Both T3 and T4 are used to treat thyroid hormone deficiency (hypothyroidism).

3. TEST PRINCIPLE

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and the enzyme-antigen conjugate for a limited number of unsolubilized binding sites. The interaction is illustrated by the following equation:

$$^{Enz}.Ag + Ag + Ab_{cw} \underset{K_{-a}}{\overset{K_a}{\rightleftharpoons}} AgAb_{cw} + {}^{Enz}.Ag\ Ab_{cw}$$

Ab_{cw}: Monospecific immobilised antibody (constant quantity)

Ag: Native Antigen (variable quantity)

Enz.Ag.: Enzyme-Antigen conjugate (constant quantity)

Ag Ab_{cw}: Antigen-Antibody complex

Enz Ag Ab_{cw}: Enzyme-antigen conjugate-Antibody complex

K_a: Rate constant of association

K_{-a}: Rate constant of disassociation

$K = k_a / k_{-a}$: Equilibrium constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a standard curve can be generated from which the antigen concentration of an unknown can be ascertained.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. 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.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants.
10. 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). Thus, total thyroxine concentration alone is not sufficient to assess clinical status. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A T3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated T4 is caused by TBG variation. A table of interfering drugs and conditions, which affect total thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.
11. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.
12. Not intended for newborn screening.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

Store all kit components at 2-8°C, do not use after expiration date. Reagents are stable for 60 days at 2-8°C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA, Heparin)

<table>
<tr><td colspan="4">The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.</td></tr>
<tr><td>Storage:</td><td>2-8°C</td><td>-20°C (Aliquots)</td><td rowspan="2">Keep away from heat or direct sun light.
Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Stability:</td><td>48 h</td><td>1 mon</td></tr>
</table>

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microplate** Ready to use. Break apart strips. Coated with Anti-T4 IgG antibody.
1 x 12 mL	ENZCONJ	**Enzyme Conjugate** Ready to use. Contains: T4 HRP Conjugate.
1 x 6 x 1 mL	CAL A-F	**Standard A-F** Ready to use. Approx. 0, 0.3, 0.95, 2.1,3.6, 7.0 ng/dL Contains: Anti-T4 IgG antibody with a preservative. Refer to vial labels for exact concentrations.
1 x 20 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (50x) Contains: 45 g/L NaCl; 55 g/L Tween-20.
1 x 15 mL	TMB SUBS	**TMB Substrate** Ready to use. Contains: 0.26 g/L H_2O_2-TMB.
1 x 15 mL	STOP	**Stop Solution** Ready to use. Contains: 0.15 mol/L sulphuric acid.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 50 and 100 µL
2. 8-Channel Micropipettor with reagent reservoirs
3. Wash bottle, automated or semi-automated microtiter plate washing system
4. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
5. Bidistilled or deionised water
6. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.
9. Each test run needs a standard curve.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated components

Dilute / dissolve	Component	with	Diluent	Relation	Storage	Stability
20 mL	**WASHBUF CONC**	980 mL	bidist. water	1:50	2-8°C	1 mon

11. TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-25°C).

1.	Pipette **50 µL** of each **Standard, Control and sample** into the respective wells of the Microtiter Plate.
2.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
3.	Cover plate with adhesive foil. Shake plate carefully for 20-30 seconds to mix and cover. **Incubate 60 minutes** at **RT (18-25°C).**
4.	Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage.
5.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
7.	**Incubate** at **RT (18-25°C)** for **15 minutes.**
8.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Color changes from blue to yellow.
9.	Mix gently for 15-20 seconds.
10.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **30 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include he 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

13. CALCULATION OF RESULTS

13.1. Interpretation of results

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.2. Note

Maximum Absorbance (A calibrator) = O.D >1.0

13.3. Mean Absorbance

Calculate the mean of the absorbance for each point of the standard curve and of each sample.

13.4. Standard Curve

Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (p.e.: Four Parameter Logistic).

13.5. Calculation of Results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/dL.

14. EXPECTED VALUES

A study of euthyroid adult population was undertaken to determine expected values for the FT4 EIA Test System. The mean values, standard deviations and expected ranges are:

	Mean (ng/dL)	SD	Range (ng/dL)
Adult	1.4	0.6	0.8-2.0
Pregnancy	1.5	0.7	0.8-2.2

15. LIMITATIONS OF THE PROCEDURE

Serum references and controls may exhibit cloudiness with time. This occurrence does not affect the accuracy or performance of the test. Use until exhausted or until expiration date on the label. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond 10 minutes to avoid assay drift. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

16. PERFORMANCE

16.1. Precision

16.1.1. Intra-Assay Variation

Within run variation was determined by replicate measurements (20x) of three different control sera in one assay. The within assay variability is ≤ 10.98%.

16.1.2. Inter Assay Variation

Between run variations was determined by replicate measurements (10x) of three different control sera in different lots of kit. The between assay variability is ≤ 10.81%.

16.2. Sensitivity

The lowest detectable concentration of FT4 that can be distinguished from the zero standard is 0.05 ng/dL at the 95 % confidence limit.

16.3. Specificity

The cross reaction of the antibody are shown in the table:

Substance	Cross-reactivity	Concentration
l -Thyroxine	1.0000	---
d -Thyroxine	0.9800	10 µg/dL
l-Triiodo-thyronine	0.0300	100 µg/dL
d-Triiodo-thyronine	0.0150	100 µg/dL
Monoiodo-Tyrosine	N/D	100 µg/mL
Diiodo-Tyrosine	N/D	100 µg/mL
Triiodothyroacetic Acid	N/D	100 µg/mL
Tetraiodothyroacetic Acid	0.0001	100 µg/mL

16.4. Correlation with RIA reference method

The FT4 Microplate was compared with a coated tube radioimmunoassay method. The total number of specimens was 197. The linear regression curve was calculated:

(FT4 RIA) = 0.952*(FT4 IBL) + 0.103
r^2 = 0.920

17. ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction
- no conjugate pipetted
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers
- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed)

CV Intra-Assay too high
- reagents and/or strips not pre-warmed to Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

CV Inter-Assay too
- incubation conditions not constant (time, temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

18. PRODUCT LITERATURE REFERENCES

1. Barker, S.B, JBC 173, 175, (1948).
2. Chopra, I.J, et al J. Clinical EndocrinoL, 33, 865 (1971)
3. Young, D.S, et al Clinical Chemistry 21, 3660 (1975)
4. Sterling, L, Cleveland CRC Press, P. 19-51 (1975)

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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