FT4 ELISA

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

REF RE55241

ʃ 96

ipi 2-8°C

EU: IVD CE
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 (unbind) - 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 TGB 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 TGB. Changes in the circulating levels of TGB 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 TGB concentration, estrogogenic hormones (pregnancy, oral contraceptives and estrogen) and drugs that bind to TGB preventing its binding to T4 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 free T4 (FT4, antigen) in the sample competes with the antigenic T4 conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti T4 coated on the microplate (solid phase).

The interaction is illustrated by the following equation:

\[
\frac{K_a}{K_a} = \frac{K_a}{K_a} \quad \text{Ag} + \text{AgAb}_{cw} + \text{EnzAgAb}_{cw} + \text{EnzAgAb}_{cw}
\]

\[
\text{Ab}_{cw}: \text{monospecific immobilised antibody (constant quantity)}
\]
\[
\text{Ag}: \text{native antigen (variable quantity)}\]
\[
\text{EnzAg}: \text{antigen conjugated to enzyme HRP (constant quantity)}\]
\[
\text{AgAb}_{cw}: \text{antigen-antibody complex}\]
\[
\text{EnzAgAb}_{cw}: \text{antigen-HRP-antibody complex}\]
\[
K_a: \text{rate constant of association}\]
\[
K_a: \text{rate constant of disassociation}\]
\[
K= \frac{k_a}{k_a}: \text{equilibrium constant}\]

After incubation, the bound/free separation is performed by a simple solid-phase washing. Then, the enzyme HRP in the bound-fraction reacts with the Substrate (H₂O₂) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H₂SO₄) is added. The color intensity is inversely proportional to the FT4 concentration of in the sample. FT4 concentration in the sample is calculated through a calibration curve.
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/H₂O₂ to directed sunlight, metals or oxidants. Do not freeze the solution.
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. 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. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.

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 reagents are stable until the expiry date when stored and handled as indicated. 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.
6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA, Heparin)
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.

<table>
<thead>
<tr>
<th>Storage:</th>
<th>2-8°C</th>
<th>-20°C (Aliquots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability:</td>
<td>48 h</td>
<td>1 month</td>
</tr>
</tbody>
</table>

Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles.

7. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Symbol</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 12 x 8</td>
<td>MTP</td>
<td>Microplate Ready to use. Break apart strips. Coated with Anti-T4 antibody.</td>
</tr>
<tr>
<td>1 x 12 mL</td>
<td>ENZCONJ</td>
<td>Enzyme Conjugate Ready to use. Contains: T4 HRP Conjugate. Avoid direct sunlight, store protected from light.</td>
</tr>
<tr>
<td>1 x 6 x 1 mL</td>
<td>CAL A-F</td>
<td>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. Concentrations are lot-specific as indicated on the bottle labels or QC Certificate.</td>
</tr>
<tr>
<td>1 x 20 mL</td>
<td>WASHBUF CONC</td>
<td>Wash Buffer, Concentrate (50x) Contains: 45 g/L NaCl; 55 g/L Tween-20.</td>
</tr>
<tr>
<td>1 x 15 mL</td>
<td>TMB SUBS</td>
<td>TMB Substrate Ready to use. Contains: 0.26 g/L H₂O₂-TMB.</td>
</tr>
<tr>
<td>1 x 15 mL</td>
<td>STOP</td>
<td>Stop Solution Ready to use. Contains: 0.15 mol/L sulphuric acid.</td>
</tr>
</tbody>
</table>

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**

**Preparation of concentrated components**

<table>
<thead>
<tr>
<th>Dilute / dissolve</th>
<th>Component</th>
<th>with</th>
<th>Diluent</th>
<th>Relation</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mL</td>
<td>WASHBUF CONC</td>
<td>980 mL bidist. water</td>
<td>1:50</td>
<td>2-8°C</td>
<td>1 month</td>
<td></td>
</tr>
</tbody>
</table>

11. **TEST PROCEDURE**

As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-25°C) for at least 30 minutes.

At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature. Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°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.**

**Important note:** During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

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** in the dark.

8. Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well.


10. **Measure** optical density with a photometer at **450 nm** (Reference wavelength of 600-650 nm) within **5 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 the 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

Mean Absorbance
Calculate the mean of the absorbance (OD) for each point of the calibration curve and of each sample.

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).

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 ELISA test. The mean values, standard deviations and expected ranges are:

<table>
<thead>
<tr>
<th></th>
<th>Mean (ng/dL)</th>
<th>SD</th>
<th>Range (ng/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>1.4</td>
<td>0.6</td>
<td>0.8-2.0</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>1.5</td>
<td>0.7</td>
<td>0.8-2.2</td>
</tr>
</tbody>
</table>

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
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

Precision

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%.

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%.

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.

Specificity
The cross-reactivity of the thyroxine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of thyroxine needed to displace the same amount of tracer:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross-reactivity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Thyroxine</td>
<td>1.0000</td>
<td>---</td>
</tr>
<tr>
<td>D-Thyroxine</td>
<td>0.9800</td>
<td>10 µg/dL</td>
</tr>
<tr>
<td>L-Triiodo-thyronine</td>
<td>0.0300</td>
<td>100 µg/dL</td>
</tr>
<tr>
<td>D-Triiodo-thyronine</td>
<td>0.0150</td>
<td>100 µg/dL</td>
</tr>
<tr>
<td>Monoiodo-Tyrosine</td>
<td>N/D</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Diodo-Tyrosine</td>
<td>N/D</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Triiodothyroacetic Acid</td>
<td>N/D</td>
<td>100 µg/mL</td>
</tr>
<tr>
<td>Tetraiodothyroacetic Acid</td>
<td>0.0001</td>
<td>100 µg/mL</td>
</tr>
</tbody>
</table>

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

\[
(\text{FT4 RIA}) = 0.952 \times (\text{FT4 IBL}) + 0.103
\]

\[\text{r}^2 = 0.920\]
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 high
- 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
### Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός Εφαρμογής:</td>
</tr>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρήση Μοναδικά:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamiento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td></td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td></td>
<td>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. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td></td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

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 συμβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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**COMPLAINTS:** Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

**WARRANTY:** The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

**LIMITATION OF LIABILITY:** IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

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