# A061-201

# DELFIA® Free Thyroxine ($FT_4$)

## Time-resolved fluoroimmunoassay

Instructions for use. Reagents for 96 assays

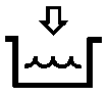
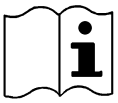
Manufactured by:
**Wallac Oy,**
**Mustionkatu 6, FI-20750 Turku, Finland**

**FOR *IN VITRO* DIAGNOSTIC USE**

CE

PerkinElmer®

**SYMBOLS**

| | |
|---|---|
| IVD | *In vitro* diagnostic medical device / Dispositif médical de diagnostic *in vitro* / *In-Vitro*-Diagnostikum / Producto sanitario para diagnóstico *in vitro* / Dispositivo medico-diagnostico *in vitro* / Dispositivo médico para diagnóstico *in vitro* / Medicinteknisk produkt för *in vitro*-diagnostik / Medicinsk udstyr til *in vitro*-diagnostik / In vitro-diagnostisk medisinsk utstyr |
| LOT | Batch code / Code du lot / Chargenbezeichnung / Código de lote / Codice del lotto / Código do lote / Lot nummer / Lotnummer / Partikode |
| PN | Packing number / Numéro d'emballage / Packnummer / Número de envase / Numero confezioni / Número de embalagem / Förpackningsnummer / Emballagenummer / Pakkenummer |
| REF | Catalog number / Référence du catalogue / Bestellnummer / Número de catálogo / Numero di catalogo / Referência de catálogo / Katalognummer / Katalognummer / Katalognummer |
|  | Use by / Utiliser jusqu'au / Verwendbar bis / Fecha de caducidad / Utilizzare entro / Prazo de validade / Använd före / Holdbar til / Brukes innen |
|  | Temperature limitation / Limites de température / Temperaturbegrenzung / Limite de temperatura / Limiti di temperatura / Limites de temperatura / Temperaturbegränsning / Temperaturbegrænsning / Temperatur-begrensning |
|  | Add liquid / Ajout de liquide / Flüssigkeit zugeben / Añadir líquido / Aggiungi liquido / Adicionar líquido / Tillsätt vätska / Tilføj væske / Tilsett væske |
|  | Store in the dark / Conserver à l'abri de la lumière / Dunkel aufbewahren / Almacenar en ambiente oscuro / Conservare al buio / Armazenar no escuro / Förvaras mörkt / Opbevares mørkt / Oppbevares mørkt |
|  | Contains sufficient for <n> tests / Contenu suffisant pour "n" tests / Inhalt ausreichend für <n> Prüfungen / Contenido suficiente para <n> ensayos / Contenuto sufficiente per "n" saggi / Conteúdo suficiente para "n" ensaios / Räcker till "n" antal tester / Indeholder tilstrækkeligt til "n" test / Innholdet rekker til <n> tester |
|  | Consult instructions for use / Consulter les instructions d'utilisation / Gebrauchsanweisung beachten / Consulte las instrucciones de uso / Consultare le istruzioni per l'uso / Consulte as instruções de utilização / Se bruksanvisningen / Se brugsanvisning / Les bruksanvisningen |
|  | Manufacturer / Fabricant / Hersteller / Fabricante / Fabbricante / Fabricante / Tillverkare / Producent / Produsent |
|  | This way up / Haut / Diese Seite oben / Este lado arriba / Questo lato in alto / Este lado para cima / Denna sida upp / Denne side op / Denne side opp |
|  | Recyclable / Recyclable / Recyclebar / Reciclable / Riciclabile / Reciclável / Återvinningsbart / Genanvendeligt / Resirkulerbar |

# DELFIA® Free Thyroxine ($FT_4$) kit

## INTENDED USE

This kit is intended for the quantitative determination of human free thyroxine ($FT_4$) in serum.

## SUMMARY AND EXPLANATION OF THE ASSAY

Thyroxine ($T_4$; 3,5,3',5'-tetraiodo-L-thyronine) and triiodothyronine ($T_3$; 3,5,3'-triiodo-thyronine) are iodine-containing hormones produced and secreted by the thyroid gland. Thyroid hormones are important regulators of the metabolic rate in the body; they accomplish this by acting as catalysts in oxidative reactions (1).

The biosynthesis of thyroid hormones involves the active accumulation of inorganic iodine in the thyroid gland. The oxidized iodine is bound to tyrosine residues of thyroglobulin, the major protein of the thyroid gland. It has been proposed that thyroxine is formed when two diiodinated tyrosine residues are linked together. Thyroxine is stored within the thyroglobulin molecule until it is proteolytically released and secreted into the circulation by exocytosis (1). Thyroxine is de-iodinated to $T_3$ in the peripheral circulation.

The release of thyroxine is regulated by a feedback mechanism along the pituitary-hypothalamic axis. Thyroid hormones inhibit the secretion of thyroid-stimulating hormone (TSH) from the pituitary gland, and decrease the responsiveness of the thyrotropes to hypothalamic TSH-releasing hormone (TRH). As a result, homeostasis of circulating thyroid hormones is maintained. Moreover, there is an autoregulatory mechanism, which ensures that the thyroid hormone pool within the thyroid gland remains constant regardless of fluctuations in the iodine content of the gland (1,2).

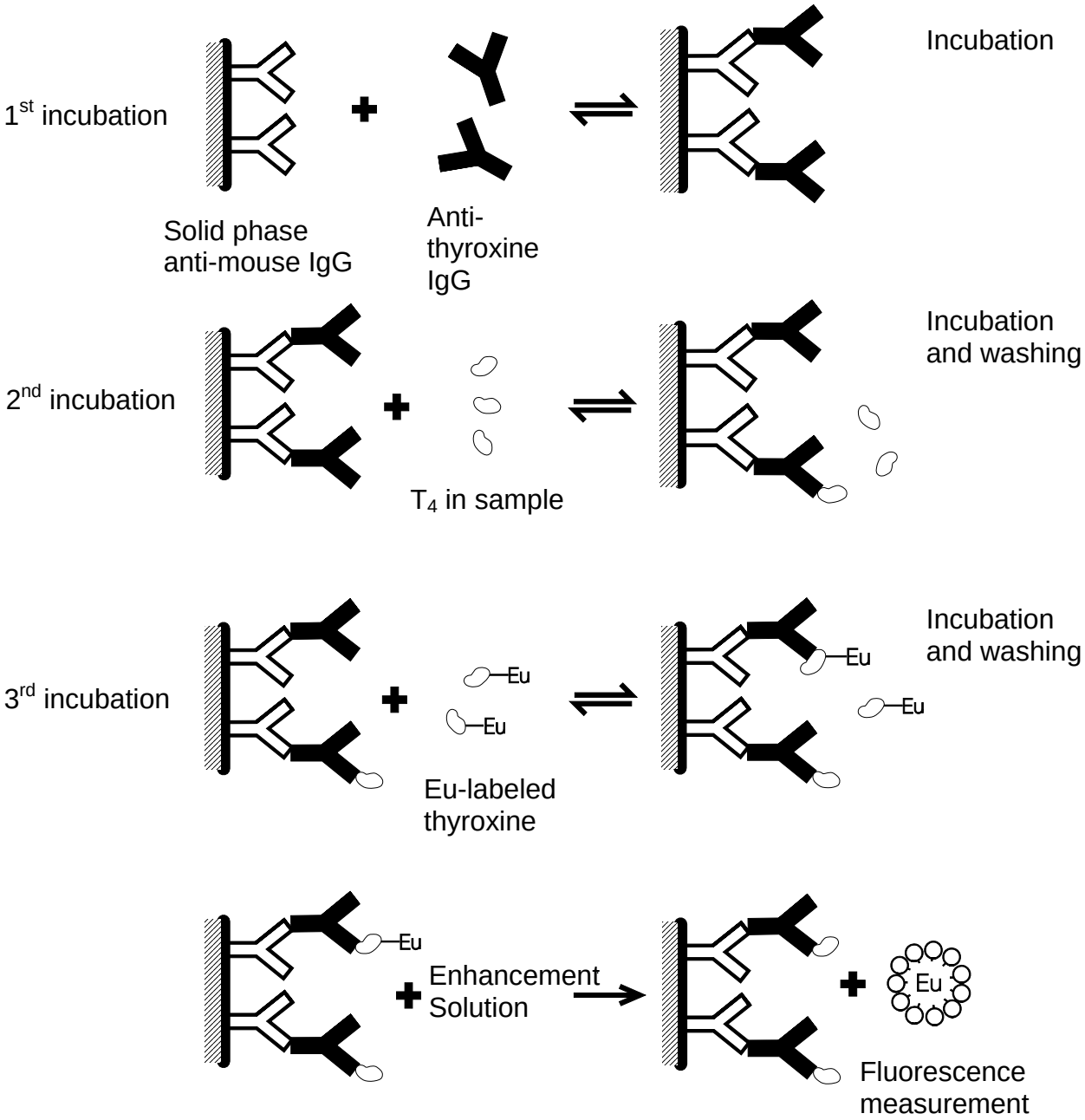
Thyroxine circulates in the blood associated with binding proteins with different affinity and capacity. About 99.97 % of the thyroxine in blood is bound to thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA) and albumin. Only a small fraction (0.03 %) of the blood thyroxine is in free form, which is considered to be the active form (1,3,4,5).

Determination of free thyroxine in serum is an important parameter in assessing thyroid function. In hypothyroidism the serum concentration is generally depressed and in hyperthyroidism the level is generally elevated (6,7). The free fraction is independent of serum proteins, unlike the bound hormone (8). Changes in binding protein concentrations and binding capacities can be caused by pregnancy, hereditary factors (familial dysalbuminemic hyperthyroxinemia), severe diseases (non-thyroid-illness), estrogen treatment, anabolic steroids or drugs (9). In such cases a more reliable indication of the metabolic situation is given by the determination of $FT_4$ rather than total thyroxine.

## PRINCIPLES OF THE ASSAY

The DELFIA® $FT_4$ assay is a solid phase time-resolved fluoroimmunoassay based on the back-titration principle, and using second-antibody separation. The anti-$T_4$ monoclonal antibody (derived from mice) is first reacted with the solid phase anti-mouse IgG. After that the sample $FT_4$ reacts with the anti-$T_4$ antibody. After the second incubation, buffer and serum are washed away, and, in a third incubation step, the remaining empty sites on the $T_4$-antibody are back titrated with Eu-labeled $T_4$ (10,11,12).

Enhancement Solution dissociates europium ions from the labeled $T_4$ into solution where they form highly fluorescent chelates with components of the Enhancement Solution. The fluorescence of each sample is inversely proportional to the concentration of $FT_4$ in the sample.




DELFIA is a registered trademark of PerkinElmer, Inc.

## KIT CONTENTS

Each DELFIA $FT_4$ kit contains reagents for 96 assays.

The expiry date of the unopened kit is stated on the outer label. Store at +2 - +8°C.

Once opened, the kit components are stable for up to 2 weeks when used as described in the section "ASSAY PROCEDURE".

### Reagents

| Component | Quantity | Shelf life and storage |
|---|---|---|
| $FT_4$ Standards (approx. values)<br><br>A 0 pmol/L<br>B 2.8 pmol/L<br>C 6.8 pmol/L<br>D 15.4 pmol/L<br>E 36 pmol/L<br>F 80 pmol/L | 6 vials, lyophilized<br><br>The exact $FT_4$ concentrations are given on the lot specific quality control certificate included in the kit. | +2 - +8°C until expiry date stated on the vial label. |

The standards are in human $T_4$-free serum. The standards have been calibrated using equilibrium dialysis.

Conversion factor: 1 pmol/L = 0.78 pg/mL = 0.078 ng/dL.

**NOTE: The powder contains sodium azide (< 1 %) as preservative and it is harmful by inhalation, in contact with skin and if swallowed.**

| | | |
|---|---|---|
| $T_4$-Eu tracer stock solution (~ 300 nmol/L) | 1 vial, 0.75 mL | +2 - +8°C until expiry date stated on the vial label. |

The tracer is in Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, and < 0.1 % sodium azide as preservative.

| | | |
|---|---|---|
| $T_4$ Antibody stock solution (~ 2 µg/mL) (mouse monoclonal) | 1 vial, 0.75 mL | +2 - +8°C until expiry date stated on the vial label. |

The antibody is in Tris-HCl buffered (pH 7.8) salt solution with gelatin, an inert blue dye, and < 0.1 % sodium azide as preservative.

| | | |
|---|---|---|
| Wash Concentrate | 1 bottle, 40 mL | +2 - +8°C until expiry date stated on the bottle label. |
| A 25-fold concentration of Tris-HCl buffered (pH 7.8) salt solution with Tween 20. Contains Germall II[1] as preservative. | | |
| $FT_4$ Assay Buffer (RED) | 1 bottle, 30 mL | +2 - +8°C until expiry date stated on the bottle label. |
| Ready-for-use Tris-HCl buffered (pH 7.4) salt solution with an inert red dye, and < 0.1 % sodium azide as preservative. | | |
| $FT_4$ Incubation Buffer (YELLOW) | 1 bottle, 30 mL | +2 - +8°C **in dark** until expiry date stated on the bottle label. |
| Ready-for-use Tris-HCl buffered (pH 7.4) salt solution with 8-anilino-1-naphthalene-sulfonic acid (ANS), diethylenetriaminepentaacetic acid (DTPA), and < 0.1 % sodium azide as preservative. | | |
| Enhancement Solution | 1 bottle, 50 mL | +2 - +8°C until expiry date stated on the bottle label. Shelf life 6 months at room temperature (+20 - +25°C). Avoid direct sunlight. |
| Ready-for-use Enhancement Solution with Triton X-100[2], acetic acid and chelators. | | |
| Anti-Mouse IgG Microtitration Strips. 8 x 12 wells coated with anti-mouse IgG (raised in rabbit) | 1 plate | +2 - +8°C until expiry date stated on the label. |
| Lot specific quality control certificate | 1 pc | |

[1] Germall is a registered trademark of ISP Investments, Inc.
[2] Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology.

## MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The DELFIA $FT_4$ kit is part of a complete system of immunodiagnostic reagents and instrumentation. The DELFIA system requires the following items, which are available from Wallac Oy or PerkinElmer, Inc. and its distributors.

1. Time-resolved fluorometer plus printer and (optional) computer
2. Automatic washer - DELFIA Platewash (prod. no. 1296-026)
3. Automatic shaker - DELFIA Plateshake (prod. no. 1296-003/004)
4. Pipette for dispensing the diluted tracer solution and the diluted antibody solution - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively DELFIA Plate Dispense with the DELFIA Dispense Unit (prod. nos. 1296-041 and 1296-043)
5. Pipette for dispensing the Enhancement Solution - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively the DELFIA Plate Dispense (prod. no. 1296-041)

In addition to the DELFIA system the following are required:

- precision pipettes for dispensing microliter volumes and pipettes for dispensing milliliter volumes
- deionized water

## SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Heparin and EDTA plasma can be used, but citrate plasma should be avoided. Hemolytic (hemoglobin $\leq$ 5 g/L) and icteric (bilirubin $\leq$ 30 mg/dL) serum samples do not interfere with the assay, but lipemia may cause variation in free thyroxine concentrations.

Samples can be stored for 2 days at +2 - +8°C. For longer periods store samples at -20°C. Repeated freezing and thawing should be avoided.

## WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

This kit should only be used by adequately trained personnel.

This kit contains reagents manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies, and found to be negative. Nevertheless all recommended precautions for the handling of blood derivatives should be observed. Please refer to the U.S. Department of Health and Human Services publication "Biosafety in Microbiological and Biomedical Laboratories" or any other local or national regulation.

Handle all patient specimens as potentially infectious.

Reagents contain sodium azide ($NaN_3$) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Lyophilized $FT_4$ standards contain sodium azide. The powder is harmful by inhalation, in contact with skin and if swallowed.

Disposal of all waste should be in accordance with local regulations.

## ASSAY PROCEDURE

Perform each determination in duplicate for both standards and unknowns. A standard curve should be run with each assay. All reagents and samples except for the Incubation Buffer (+4 - +8°C) must be brought to room temperature (+20 - +25°C) before use.

| 1. Preparation of reagents | Reconstituted stability |
|---|---|
| $FT_4$ standards | 4 weeks at +2 - +8°C. |

Add exactly 1.1 mL of deionized water to each vial and mix gently. Allow to stand for at least 30 minutes before use.

**NOTE: The powder contains sodium azide (< 1 %) as preservative and it is harmful by inhalation, in contact with skin and if swallowed. The dissolved standards contain < 0.1 % sodium azide and are not considered harmful.**

| | |
|---|---|
| Wash solution | 2 weeks at +2 - +25°C<br>in a sealed container. |

Pour the 40 mL of Wash Concentrate into a clean container and dilute 25-fold by adding 960 mL of deionized water to give a buffered wash solution (pH 7.8).

| | |
|---|---|
| $FT_4$ antibody solution | **Prepare within one hour of use.** |

Prepare the needed volume of antibody dilution by mixing 30 µL of antibody stock solution with 3 mL of $FT_4$ Assay Buffer per strip (see table in the Summary Protocol Sheet). ANTIBODY SOLUTION CONTAINING ASSAY BUFFER IS RED.

$FT_4$ tracer solution **Prepare within one hour of use, during the second incubation.**

Prepare the needed volume of tracer dilution by mixing 30 µL of tracer stock solution with 3 mL of $FT_4$ Incubation Buffer per strip (see table in the Summary Protocol Sheet). TRACER SOLUTION CONTAINING INCUBATION BUFFER IS YELLOW.

**It is recommended that cold $FT_4$ Incubation Buffer (+4 - +8°C) is used when preparing and dispensing the $FT_4$ tracer solution.**

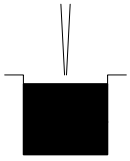
**It is important that the Incubation Buffer does not come into contact with tracer stock solution not intended for immediate use.**

We advise the use of disposable plastic containers to prepare the antibody and tracer working solutions.

2. Transfer the required number of microtitration strips to a strip frame.

   Note: Open the foil from three sides only and fold it aside leaving the plate-specific information on the package. Return the remaining strips into the package and press the foil cover back on as tightly as possible. Leave the desiccant in the package. Alternatively, store the remaining strips in a resealable plastic bag with the desiccant.

3. Pipette 200 µL of diluted antibody solution (red) to each well using **the recommended Eppendorf Multipette** after discarding the first aliquot, or use the DELFIA Dispense Unit.



4. Incubate the frame for at least 70 minutes at room temperature with **slow** shaking on the DELFIA Plateshake. Do not incubate longer than 80 minutes. Do not aspirate the diluted antibody solution.

5. Pipette 25 µL of the $FT_4$ Standards (Std) and patient serum specimens (unknowns - Unk) onto the antibody solution. The following plate map is given as an example. Each laboratory can decide on the best positioning of the controls and samples.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Strip |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Std A | Std A | Std B | Std B | Std C | Std C | Std D | Std D | Std E | Std E | Std F | Std F | A |
| 1st Unk | 1st Unk | 2nd Unk | 2nd Unk | 3rd Unk | 3rd Unk | etc. | | | | | | B |
| | | | | | | | | | | | | C etc. |

6. Incubate the frame for 60 minutes (± 10 minutes) at room temperature with **slow** shaking on the DELFIA Plateshake.

7. After the second incubation step, aspirate and wash each strip with the DELFIA Platewash using program 61 (wash 1).

8. Add 200 µL of cold tracer dilution (yellow) to each well. Pipetting should be as for the antibody solution in step 3 above.

9. Incubate the frame for 30 minutes (± 5 minutes) **without shaking** at +4°C (max +8°C). It is important that this incubation is carried out at the correct temperature.

10. After the tracer incubation step, aspirate and wash each strip with the DELFIA Platewash using program 61 (wash 2).

11. Add 200 µL of Enhancement Solution directly from the reagent bottle to each well using **the recommended Eppendorf Multipette** after flushing the Combitip once with Enhancement Solution (to waste), or use the DELFIA Plate Dispense. Refill the Combitip and discard the first aliquot. Avoid touching the edge of the well or its contents.

12. Shake the frame **slowly** for 5 minutes. The fluorescence is stable for several hours if evaporation is prevented. However, we recommend measurement within 1 hour as external factors may cause a decrease in signal with time, although this is extremely rare.

13. Ensure that each strip is firmly seated in the frame and measure the fluorescence in the time-resolved fluorometer.

    When using the 1232 or 1234 fluorometer select kit program 61 or MultiCalc® [3] protocol "61 FT4" for automatic measurement and result calculation.

    When using VICTOR[2] D start the measurement from the Start Wizard, select "FT4" from Protocols/Kits panel "Thyroid" and define the number of plates and samples.

    Check the parameter group for program 61 or the MultiCalc protocol "61 FT4". If you change the replicate number for the unknowns please change the protocol accordingly (see fluorometer manual or MultiCalc manual for editing the parameters).

[3] MultiCalc is a registered trademark of PerkinElmer, Inc.
VICTOR is a trademark of PerkinElmer, Inc.

| | | | |
|---|---|---|---|
| ASSAY TYPE | : | FIA | |
| FITTING METHOD | : | SPLINE SMOOTHED | |
| X-AXIS | : | LOGARITHMIC | |
| Y-AXIS | : | $B/B_{max}$ | |
| BLANKS | : | 0 | |
| STANDARDS | : | 6 | |
| STANDARD REPLICATES | : | 2 | |
| STANDARD CONC | : | A | **(Make sure that the $FT_4$ standard** |
| STANDARD CONC | : | B | **concentrations correspond to those** |
| STANDARD CONC | : | C | **given on the lot specific quality control** |
| STANDARD CONC | : | D | **certificate. If this is not the case, enter** |
| STANDARD CONC | : | E | **the new concentrations.)** |
| STANDARD CONC | : | F | |
| UNKNOWN REPLICATES | : | 2 | |

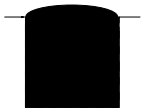
## PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the DELFIA kit. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use kit reagents after the expiry date printed on the kit label.

2. Any deviation from the assay procedure may affect the results.

3. Reagents except for the Incubation Buffer (+4 - +8°C) should be allowed to reach room temperature (+20 - +25°C) prior to sample preparation. Frozen specimens should be brought to room temperature slowly and gently mixed by hand. Do not vigorously vortex or mix patient specimens.

4. When washing the strips, ensure that each well is filled up completely to the top edge as shown in the figure. After washing the strips, check that the wells are dry. If there is moisture left, invert the plate and tap firmly against absorbent paper.



   For detailed information on the cleaning and maintenance of the washing device, please refer to the DELFIA Platewash manual.

5. The avoidance of europium contamination and resulting high fluorescent background demands high standard pipetting and washing techniques. Thus it is extremely important to use the pipettes supplied with the DELFIA system for the recommended purposes only.

   The Enhancement Solution should be dispensed using only the recommended Eppendorf Multipette after the Combitip has been first flushed with Enhancement Solution according to the Directions for Use. The same Combitip must not be used for pipetting any other reagent. After use place the Eppendorf Multipette on the pipette stand, with the Combitip still attached.

   When using the DELFIA Plate Dispense and DELFIA Dispense Unit, please refer to the manual.

## CALCULATION OF RESULTS

The DELFIA system incorporates programs for data reduction, and the results are obtained as printouts of standard curves, unknown concentrations etc. (see Fluorometer instrument manual or MultiCalc manual for detailed information).

### Quality control

The use of control sera is advised to assure the day-to-day validity of results. The controls should be run in the same way as the samples. It is recommended that the laboratory prepares its own serum pools at different levels, or alternatively uses commercial controls, e.g. Lyphochek[4]. A high, a medium and a low level control should be run in each assay; if the assay includes more than one plate, controls should be run on each plate. Patient results should only be reported if control results for the assay meet the laboratory's established criteria for acceptability (14).

We also recommend participation in external quality control schemes.

## LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definite clinical judgement should not be based on the results of any single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

Hemolytic (hemoglobin $\leq$ 5 g/L) and icteric (bilirubin $\leq$ 30 mg/dL) serum samples do not interfere, but lipemia may cause variation in free thyroxine concentrations.

Please also refer to the section "PROCEDURAL NOTES".

## EXPECTED VALUES[5] AND INTERPRETATION OF RESULTS

Please note that the values mentioned in this section should only be used as a guideline, and each laboratory should establish its own reference range.

The reference range when measured from 79 apparently healthy men (aged 23 - 58 years) and 123 apparently healthy women (aged 22 - 58 years) with the A061-201 DELFIA $FT_4$ assay was 9.8 - 16.8 pmol/L (0.76 - 1.31 ng/dL). The mean value was 12.7 pmol/L (0.99 ng/dL).

The lower and upper extremes of the reference range were examined and their confidence intervals were estimated according to IFCC recommended non-parametric statistical treatment (13).

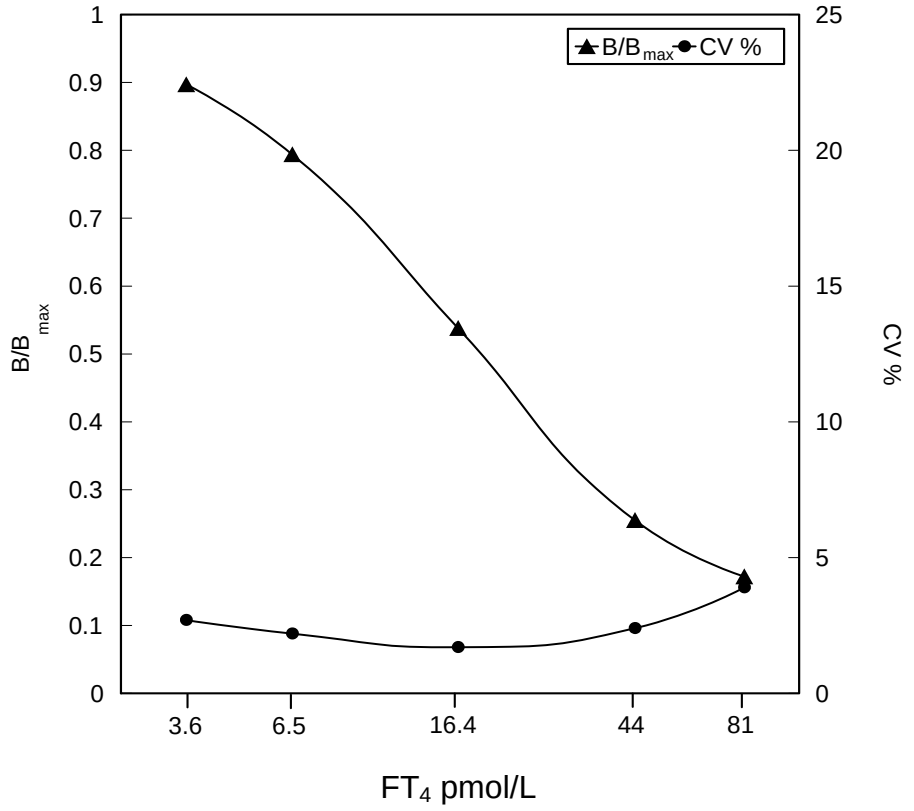
[4] Lyphochek is a registered trademark of Bio-Rad Laboratories Inc.
[5] Study performed at Wallac Oy, Turku, Finland.

Estimates:

| Fraction | Reference limit | 0.90-confidence interval |
|---|---|---|
| 2.5 % | 9.8 pmol/L (0.76 ng/dL) | 8.7 - 10.1 pmol/L (0.68 - 0.79 ng/dL) |
| 97.5 % | 16.8 pmol/L (1.31 ng/dL) | 15.9 - 17.4 pmol/L (1.24 - 1.36 ng/dL) |

## ANALYTICAL PERFORMANCE CHARACTERISTICS

A typical standard curve and precision profile obtained with the A061-201 DELFIA $FT_4$ assay are shown below. The precision profile was calculated from 336 duplicate measurements of standards and serum specimens using the MultiCalc data management program.




**Precision[6]:** The variation of the A061-201 DELFIA $FT_4$ assay was determined in 27 runs with 3 replicates using 3 DELFIA systems, and the analysis of variance approach was used to calculate the following variations:

| Serum sample | Total mean value pmol/L | Total mean value ng/dL | Intra-assay variation (% CV) | Inter-assay variation (% CV) | Total variation (% CV) |
|---|---|---|---|---|---|
| 1 | 9.8 | 0.76 | 3.7 | 6.1 | 7.1 |
| 2 | 17.1 | 1.33 | 3.0 | 4.1 | 5.1 |
| 3 | 20.9 | 1.63 | 3.3 | 4.0 | 5.1 |

[6] Study performed at Wallac Oy, Turku, Finland.

**Analytical sensitivity[7]:** The analytical sensitivity of the A061-201 DELFIA $FT_4$ assay is typically better than 2 pmol/L (0.16 ng/dL), if the analytical sensitivity is defined as the value which is 2 standard deviations below the mean of the zero standard measurement values (mean value - 2 SD) (n = 72).

**Method comparison[8]:**

The A061-201 DELFIA Free Thyroxine ($FT_4$) kit (y) was compared with the 1244-061 DELFIA Free Thyroxine ($FT_4$) kit (x) using patient specimens in the range of 4.5 - 84.3 pmol/L (0.35 - 6.58 ng/dL) free thyroxine. The correlation was found to be:

$y = 0.98x + 0.27$; $r = 1.00$; $(n = 322)$

The 1244-061 DELFIA Free Thyroxine ($FT_4$) kit (y) was compared with a commercially available analog free thyroxine radioimmunoassay kit (x). The correlation was found to be:

$y = 0.97x - 0.12$; $r = 0.94$; $(n = 100)$

The A061-201 DELFIA Free Thyroxine ($FT_4$) kit (y) was compared with the B061-201 AutoDELFIA Free Thyroxine ($FT_4$) kit (x) using patient specimens in the range of 4.5 - 84.3 pmol/L (0.35 - 6.58 ng/dL) free thyroxine. The correlation was found to be:

$y = 1.00x - 0.30$; $r = 1.00$; $(n = 322)$

**Cross reactivity[9]:** The cross reactivity (at the 50 % displacement level) was determined by setting up an assay for total $T_4$ with the antibody and tracer used in this kit.

| Substance | Cross reactivity % |
|---|---|
| D-Thyroxine | 30.1 |
| 3,3',5-Triiodo-L-thyronine ($LT_3$) | 0.89 |
| 3,3',5-Triiodoacetic acid | 0.45 |
| 3,3',5-Triiodo-D-thyronine ($DT_3$) | 0.37 |
| 3,5-Diiodo-L-thyronine | < 0.1 |
| 3,5-Diiodotyrosine (DIT) | < 0.1 |
| 5,5-Diphenylhydantoin | < 0.1 |
| 3-Iodo-L-tyrosine (MIT) | < 0.1 |
| Phenylbutazone | < 0.1 |
| 6-n-Propyl-2-thiouracil | < 0.1 |
| Methimazole | < 0.1 |
| L-Tyrosine | < 0.1 |
| 3,3-Methylene-bis (4-hydroxycoumarin) | < 0.1 |
| Acetylsalicylic acid | < 0.1 |

[7] Study performed at Wallac Oy, Turku, Finland.
[8] as above
[9] as above

**WARRANTY**

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by the manufacturer may affect the results, in which event Wallac Oy and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

Wallac Oy, its affiliates and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

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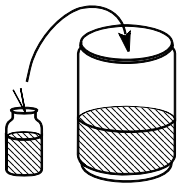
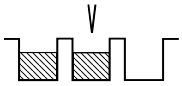
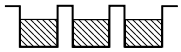
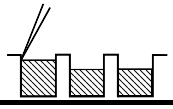
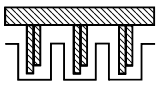
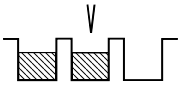
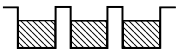
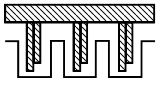
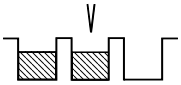
Last revision March 2011

# DELFIA® $FT_4$ kit

## Summary Protocol Sheet

<table>
<tr><td>Reconstitute standards</td><td>  </td><td colspan="5">1.1 mL deionized water,<br>30 min.</td></tr>
<tr><td>Dilute antibody solution<br>(see table)</td><td>  </td><td>Strips</td><td>Antibody stock sol. (µL)</td><td>Assay Buffer (mL)</td><td>Tracer stock sol. (µL)</td><td>Incubation Buffer (+4°C) (mL)</td></tr>
<tr><td></td><td></td><td>1</td><td>30</td><td>3</td><td>30</td><td>3</td></tr>
<tr><td></td><td></td><td>2</td><td>60</td><td>6</td><td>60</td><td>6</td></tr>
<tr><td></td><td></td><td>3</td><td>90</td><td>9</td><td>90</td><td>9</td></tr>
<tr><td></td><td></td><td>4</td><td>120</td><td>12</td><td>120</td><td>12</td></tr>
<tr><td></td><td></td><td>5</td><td>150</td><td>15</td><td>150</td><td>15</td></tr>
<tr><td></td><td></td><td>6</td><td>180</td><td>18</td><td>180</td><td>18</td></tr>
<tr><td></td><td></td><td>7</td><td>210</td><td>21</td><td>210</td><td>21</td></tr>
<tr><td></td><td></td><td>8</td><td>240</td><td>24</td><td>240</td><td>24</td></tr>
<tr><td>Dispense antibody dilution (red)</td><td>  </td><td colspan="5">200 µL/well</td></tr>
<tr><td>Incubate</td><td>  </td><td colspan="5">70 min. (+ 10 min.)<br>slow shaking at RT</td></tr>
<tr><td>Add standards and unknowns</td><td>  </td><td colspan="5">25 µL</td></tr>
<tr><td>Incubate</td><td>  </td><td colspan="5">60 min. (± 10 min.)<br>slow shaking at RT</td></tr>
<tr><td>Dilute tracer solution</td><td>(+4°C)</td><td colspan="5">see table</td></tr>
<tr><td>Wash</td><td>  </td><td colspan="5">Program 61 (x 1)</td></tr>
<tr><td>Add tracer dilution (yellow)</td><td>  </td><td colspan="5">200 µL</td></tr>
<tr><td>Incubate</td><td>  </td><td colspan="5">30 min. at +4°C</td></tr>
<tr><td>Wash</td><td>  </td><td colspan="5">Program 61 (x 4)</td></tr>
<tr><td>Enhance</td><td>  </td><td colspan="5">200 µL,<br>5 min. slow shaking</td></tr>
<tr><td>Count</td><td>  </td><td colspan="5">KIT 61<br>(check concentrations<br>from QC certificate)</td></tr>
</table>