1244-030

DELFIA®
Thyroxine (T_4)

Time-resolved fluoroimmunoassay

Instructions for use. Reagents for 96 assays

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

FOR RESEARCH USE ONLY.
Not for use in diagnostic procedures.

PerkinElmer®

SYMBOLS

Batch code

Packing number

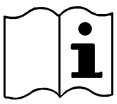
Catalog number

Use by

Temperature limitation

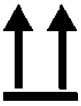
Store in the dark

Contains sufficient for <n> tests



Consult instructions for use

Manufacturer



This way up

Recyclable

DELFIA® Thyroxine (T_4) kit

APPLICATION

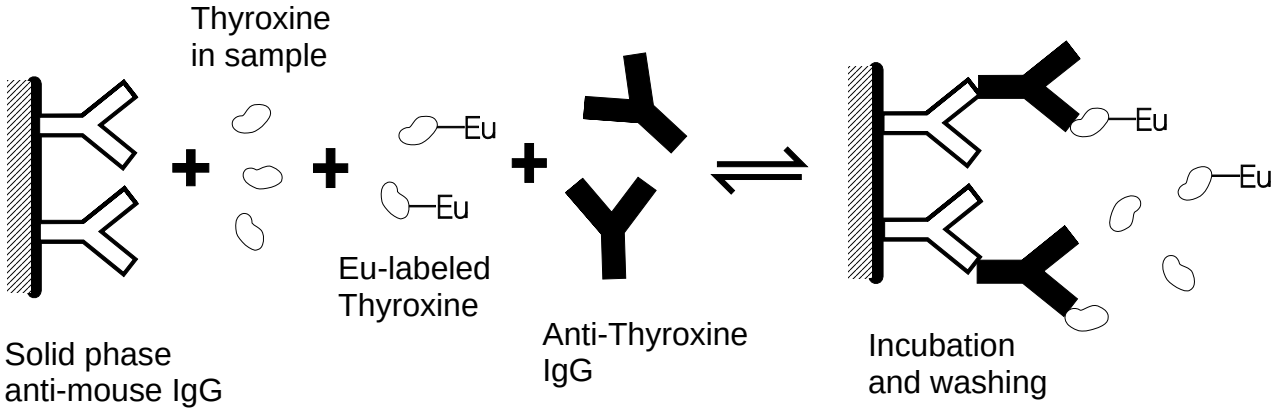
This kit is for the quantitative determination of total thyroxine (T_4) in serum.

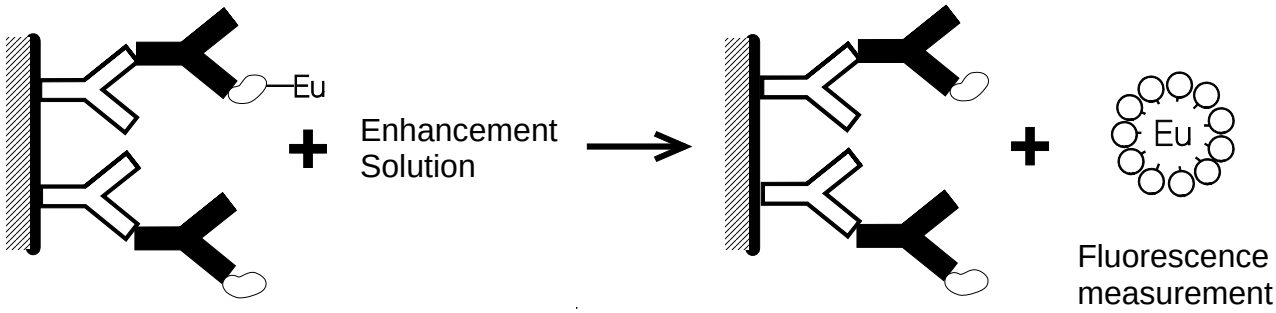
For research use only. Not for use in diagnostic procedures.

PRINCIPLES OF THE ASSAY

The DELFIA® T_4 assay is a solid phase time-resolved fluoroimmunoassay based on the competitive reaction between europium-labeled T_4 and sample T_4 for a limited amount of binding sites on T_4 specific monoclonal antibodies (derived from mice). The use of 8-anilino-1-naphthalenesulfonic acid (ANS) and salicylate in the assay buffer facilitates the release of T_4 from the binding proteins (1,2). Thus the assay measures the total amount of T_4 in the test specimen. A second antibody, directed against mouse IgG, is coated to the solid phase, and binds the IgG-thyroxine complex, giving convenient separation of the antibody-bound and free antigen.

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 in each well is then measured. The fluorescence is inversely proportional to the quantity of T_4 in the sample (3,4).





DELFIA is a registered trademark of PerkinElmer, Inc.

DELFIA is a registered trademark of PerkinElmer, Inc.

KIT CONTENTS

Each DELFIA Thyroxine 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
T_4 Standards (approx. values)	6 vials, 1.0 mL	+2–+8 °C until expiry date stated on the vial label.
A 0 nmol/L 0 µg/dL B 20 nmol/L 1.55 µg/dL C 50 nmol/L 3.89 µg/dL D 100 nmol/L 7.77 µg/dL E 150 nmol/L 11.66 µg/dL F 300 nmol/L 23.31 µg/dL	The exact T_4 concentrations are given on the lot specific quality control certificate included in the kit.	

The ready-for-use standards are in human T_4-free serum with < 0.1 % sodium azide as preservative. Conversion factor: 100 nmol/L = 7.77 µg/dL.
The standards have been calibrated using gravimetric and spectrophotometric methods.

T_4-Eu tracer stock solution (~ 150 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 (~ 12 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 bovine serum albumin, 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.

[1] Germall is a registered trademark of ISP Investments, Inc.

T_4 Assay Buffer	1 bottle, 30 mL	+2–+8 °C in dark until expiry date stated on the bottle label.
Ready-for-use Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, bovine globulin, Tween 40, 8-anilino-1-naphthalenesulfonic acid (ANS), sodium salicylate, rabbit IgG, an inert red dye, 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. Make sure that the plastic tray pack remains sealed.
Lot specific quality control certificate	1 pc	

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The DELFIA T_4 kit is part of a complete system of 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/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

[2] Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Plasma containing EDTA or citrate cannot be used due to chelating effects on europium. Heparin plasma however can be used. Hemolytic, lipemic and icteric serum samples do not interfere.

Samples can be stored 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 research use only. Not for use in diagnostic procedures.

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 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.

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 must be brought to room temperature (+20–+25 °C) before use.

1. Preparation of reagents	Reconstituted stability
Wash solution	2 weeks at +2 - +25°C 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).

T_4 tracer/antibody solution	**Prepare within one hour of use.**

Prepare the needed volume of tracer/antibody solution by mixing 3 mL T_4 Assay Buffer with 30 µL tracer stock solution and 30 µL antibody stock solution per strip (see table

in the Summary Protocol Sheet). Use clean pipette tips for pipetting tracer stock solution and antibody stock solution.

It is important that the T_4 Assay Buffer does not come into contact with tracer stock solution not intended for immediate use.

We advise the use of a disposable plastic container to prepare the tracer/antibody working solution.

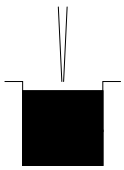
---

2. Separate the strips to be used by cutting through the protective tape with a sharp knife and transfer them to a strip frame. Pull off the protective tape from the strips intended for use. (Return the remaining strips to the plastic tray pack and reseal.) Wash each strip with the DELFIA Platewash using program 30 (prewash). Do not wash more strips than can be easily handled within 30 minutes. Ensure that no wash solution is left in the wells after washing. **Remove any remaining moisture by blotting the plate on absorbent paper.**

3. Pipette 25 µL of the T_4 Standards (Std) and serum specimens (unknowns - Unk) into the strip wells. 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.

4. Add 200 µL of diluted tracer/antibody solution to each well using **the recommended Eppendorf Multipette** after discarding the first aliquot, or use the DELFIA Dispense Unit. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or the surface of the liquid.



5. Incubate the frame for 90 minutes at room temperature with **slow** shaking using the DELFIA Plateshake. Do not incubate longer than 2 hours.

6. After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 30 (wash).

7. 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.

8. 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.

9. 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 30 or MultiCalc® [3] protocol "30 T4" for automatic measurement and result calculation.

   When using VICTOR[2] D start the measurement from the Start Wizard, select "T4" from Protocols/Kits panel "Thyroid" and define the number of plates and samples.

   Check the parameter group for program 30 or the MultiCalc protocol "30 T4". If you change the replicate number for the unknowns please change the protocol accordingly (see fluorometer manual or MultiCalc manual for editing the parameters):

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	
STANDARD CONC	:	B	**(Make sure that the T_4 standard**
STANDARD CONC	:	C	**concentrations and unit correspond to**
STANDARD CONC	:	D	**those given on the lot specific quality**
STANDARD CONC	:	E	**control certificate. If this is not the case,**
STANDARD CONC	:	F	**enter the new concentrations and if necessary change the unit in the MultiCalc protocol.)**
UNKNOWN REPLICATES	:	2	

PROCEDURAL NOTES

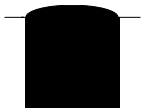
1. A thorough understanding of this package insert is necessary for successful use of the DELFIA kit. The reagents supplied with this kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use kit reagents after the expiry date printed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

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

3. Reagents 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 specimens.

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

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).

LIMITATIONS OF THE PROCEDURE

For research use only. Not for use in diagnostic procedures.

Please also refer to the section "PROCEDURAL NOTES".

REFERENCES

1. Ratcliffe, W.A., Ratcliffe, J.G., McBride, A.D., Harland, W.A and Randall, T.W. (1974): The radioimmunoassay of thyroxine in unextracted human serum. Clin. Endocrinol. **3**, 481.

2. Hüfner, M. and Hesch, R-D. (1973): A comparison of different compounds for TBG blocking used in radioimmunoassay for tri-iodothyronine. Clin. Chim. Acta **44**, 101-107.

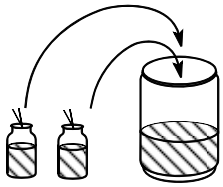
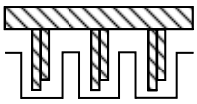
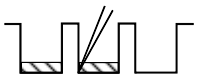
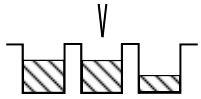
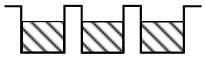
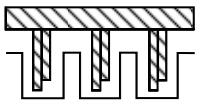
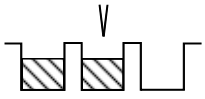
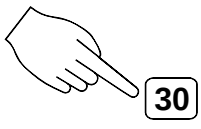
3. Soini, E. and Kojola, H. (1983): Time-resolved fluorometer for lanthanide chelates - a new generation of non-isotopic immunoassays. Clin. Chem. **29**, 65-68.

4. Hemmilä, I., Dakubu, S., Mukkala, V-M., Siitari, H. and Lövgren, T. (1984): Europium as a label in time-resolved immunofluorometric assays. Anal. Biochem. **137**, 335-343.

February 22, 2012

DELFIA® T_4 kit

Summary Protocol Sheet

<table>
<tr>
<td>Dilute tracer and antibody solution (see table)</td>
<td>  </td>
<td colspan="4">
<table>
<tr><th>Strips</th><th>Tracer stock solution (µL)</th><th>Antibody stock solution (µL)</th><th>Buffer (mL)</th></tr>
<tr><td>1</td><td>30</td><td>30</td><td>3</td></tr>
<tr><td>2</td><td>60</td><td>60</td><td>6</td></tr>
<tr><td>3</td><td>90</td><td>90</td><td>9</td></tr>
<tr><td>4</td><td>120</td><td>120</td><td>12</td></tr>
<tr><td>5</td><td>150</td><td>150</td><td>15</td></tr>
<tr><td>6</td><td>180</td><td>180</td><td>18</td></tr>
<tr><td>7</td><td>210</td><td>210</td><td>21</td></tr>
<tr><td>8</td><td>240</td><td>240</td><td>24</td></tr>
</table>
</td>
</tr>
<tr><td>Wash</td><td>  </td><td colspan="4">Program 30 (x 1)</td></tr>
<tr><td>Add standards and unknowns</td><td>  </td><td colspan="4">25 µL</td></tr>
<tr><td>Add tracer and antibody dilution</td><td>  </td><td colspan="4">200 µL</td></tr>
<tr><td>Incubate</td><td>  </td><td colspan="4">90 min. slow shaking at RT</td></tr>
<tr><td>Wash</td><td>  </td><td colspan="4">Program 30 (x 4)</td></tr>
<tr><td>Enhance</td><td>  </td><td colspan="4">200 µL,
5 min. slow shaking</td></tr>
<tr><td>Count</td><td> 
</td><td colspan="4">KIT 30
(check concentrations from QC certificate)</td></tr>
</table>