

1244-030

DELFINA[®]

Thyroxine (T₄)

Time-resolved fluoroimmunoassay

Instructions for use. Reagents for 96 assays

Manufactured by:
**PerkinElmer Life and Analytical Sciences, Wallac Oy,
Mustionkatu 6, Turku, Finland**

FOR *IN VITRO* DIAGNOSTIC USE

CE


PerkinElmer[®]
precisely.

SYMBOLS



For *in vitro* diagnostic use / Pour usage diagnostique *in vitro* / Zur *in vitro* – Diagnostik / Para diagnóstico *in vitro* / Per uso diagnostico *in vitro* / Para uso em diagnóstico *in vitro* / För användning vid *in vitro* -diagnostik / Til *in vitro* diagnostisk brug



Lot no. / Lot n° / Ch.-Nr. / N° de lote / No. lotto / No. lote / Lot nr. / Lot. nr.



Packing no. / N° d'emballage / Pack-Nr. / N° de embalaje / No. confezione / No. de empacotamento / Pack nr. / Pakke nr.



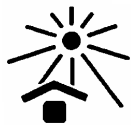
Product no. / Produit n° / Produkt-Nr. / N° de referencia / Prodotto n. / No. do produto / Produkt nr. / Produkt nr.



Expiry date / Date de péremption / Verfallsdatum / Fecha de caducidad / Data di scadenza / Data de validade / Utgångsdatum / Udløbsdato



Store at / Stocker entre / Lagerung bei / Almacenar a / Conservare a / Estocar a / Förvaras i / Opbevares ved



Protect from heat and light / A protège de la chaleur et de la lumière / Vor Wärme- und Lichteinwirkung schützen / Proteger del calor y de la luz / Tenere al riparo dal calore e dalla luce / Mantenha ao abrigo do calor e da luz / Skyddas mot värme och ljus / Beskyttes mod varme og lys



Contains reagents for 96 tests / Contient des réactifs pour 96 dosages / Enthält Reagenzien für 96 Bestimmungen / Contiene reactivos para 96 ensayos / Contiene reagenti per 96 dosaggi / Contém reagentes para 96 determinações / Innehåller reagens för 96 bestämningar / Indeholder reagenser til 96 bestemmelser



Note: Read the instructions for use / Remarque: Lire le mode d'emploi / Hinweis: Gebrauchsinformationen beachten / Nota: Leer las instrucciones de uso / Nota: Leggere le istruzioni d'uso / Nota: Leia as instruções para uso / OBS: Läs instruktioner för handhavande / Bemærk: Læs forskriften



Manufacturer / Fabricant / Hersteller / Fabricante / Produttore / Produzido por / Tillverkare / Producent

DELFLIA[®] Thyroxine (T₄) kit

INTENDED USE

This kit is intended for the quantitative determination of total human thyroxine (T₄) in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

Thyroxine (T₄; 3,5,3',5'-tetraiodo-L-thyronine) and triiodothyronine (T₃; 3,5,3'-triiodo-L-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 is 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 T₃ 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 80 % of the thyroxine in blood is bound to thyroxine-binding globulin (TBG), the rest mostly to thyroxine-binding prealbumin and a small amount to albumin. Only a small fraction (0.03 %) of the blood thyroxine is in free form, but is considered to be the active form (1,3,4,5).

Determination of total thyroxine in serum is an important parameter in assessing thyroid function. In hypothyroidism the serum concentration of thyroxine is generally depressed. In hyperthyroidism the thyroxine level is elevated except in T₃-thyrotoxicosis, where normal T₄ concentrations are found in combination with elevated T₃-concentrations (1,6,7,8).

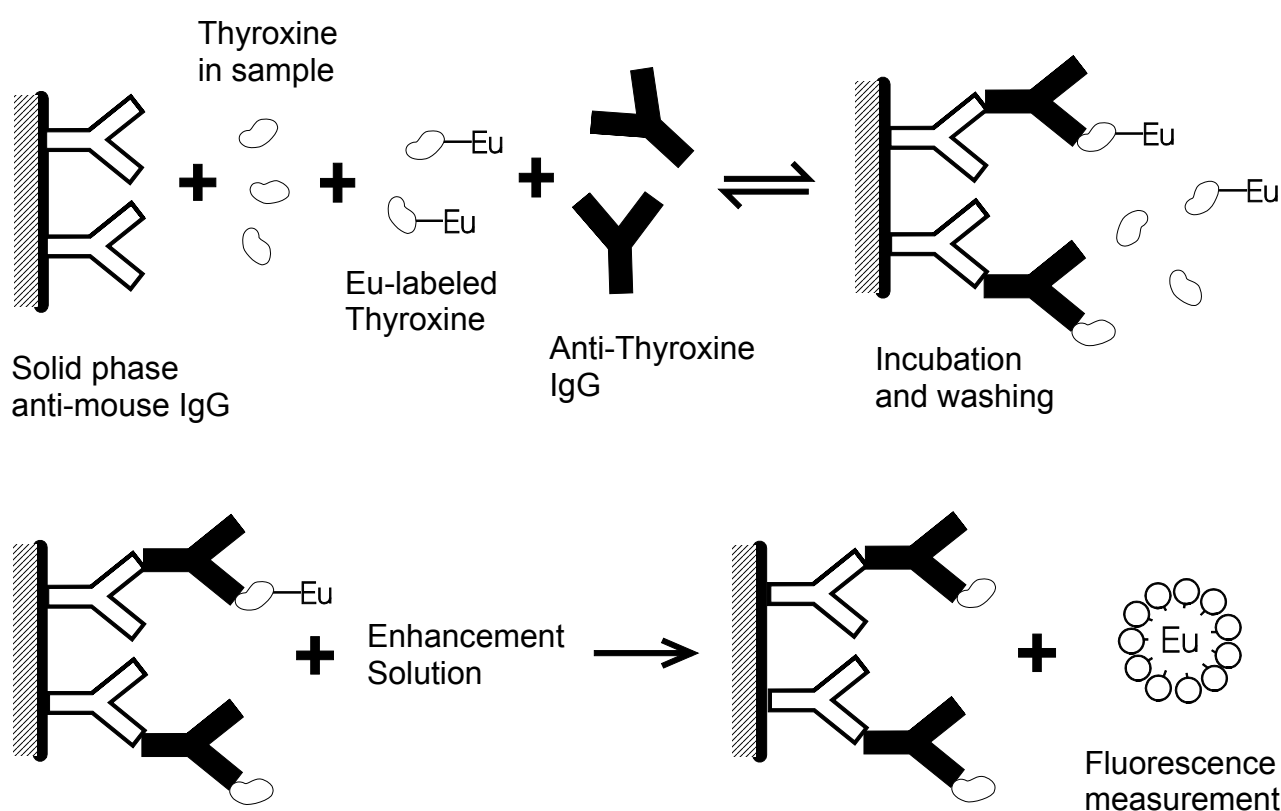
When assessing thyroid function based on total thyroxine measurement, it is important to note the clinical conditions where TBG concentrations vary.

Thyroxine concentrations in men and non-pregnant women are almost the same. During the early stages of pregnancy the thyroxine concentration increases and remains high throughout the remainder of pregnancy due to an elevated level of TBG in the blood (1).

PRINCIPLES OF THE ASSAY

The DELFIA® T₄ assay is a solid phase time-resolved fluoroimmunoassay based on the competitive reaction between europium-labeled T₄ and sample T₄ for a limited amount of binding sites on T₄ 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 T₄ from the binding proteins (9,10). Thus the assay measures the total amount of T₄ 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 T₄ 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 T₄ in the sample (11,12).



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		The exact T ₄ concentrations are given on the lot specific quality control certificate included in the kit.
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 ready-for-use standards are in human T₄-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.
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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.
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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.
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A 25-fold concentration of Tris-HCl buffered (pH 7.8) salt solution with Tween 20. Contains Germall II¹ as preservative.

T ₄ Assay Buffer	1 bottle, 30 mL	+2 - +8°C in dark until expiry date stated on the bottle label.
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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.

¹ Germall is a registered trademark of Sutton Laboratories Inc.

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.
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Ready-for-use Enhancement Solution with Triton X-100², 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.
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Lot specific quality control certificate	1 pc
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MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The DELFIA T₄ kit is part of a complete system of immunodiagnostic reagents and instrumentation. The DELFIA system requires the following items, which are available from PerkinElmer Life and Analytical Sciences or 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 Multipipette (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 Multipipette (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
- pipettes for dispensing the milliliter volumes of T₄ Assay Buffer required to prepare the tracer/antibody dilution
- distilled water

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.

² Triton is a registered trademark of Rohm and Haas Co.

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 *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 (NaN₃) 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 distilled water to give a buffered wash solution (pH 7.8).

T₄ tracer/antibody solution

Prepare within one hour of use.

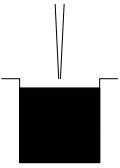
Prepare the needed volume of tracer/antibody solution by mixing 3 mL T₄ Assay Buffer with 30 µL tracer stock solution and 30 µL antibody stock solution per strip (see table in the Summary Protocol Sheet). Use clean pipette tips for pipetting tracer stock solution and antibody stock solution.

It is important that the T₄ 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.

- 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.**
- Pipette 25 µL of the T₄ Standards (Std) and patient 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.

- 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. 
- Incubate the frame for 90 minutes at room temperature with **slow** shaking using the DELFIA Plateshake. Do not incubate longer than 2 hours.
- After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 30 (wash).
- 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 VICTOR² 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):

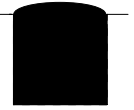
ASSAY TYPE	:	FIA	
FITTING METHOD	:	SPLINE SMOOTHED	
X-AXIS	:	LOGARITHMIC	
Y-AXIS	:	B/B _{max}	
BLANKS	:	0	
STANDARDS	:	6	
STANDARD REPLICATES	:	2	
STANDARD CONC	:	A	
STANDARD CONC	:	B	(Make sure that the T₄ standard concentrations correspond to those given on the lot specific quality control certificate. If this is not the case, enter the new concentrations.)
STANDARD CONC	:	C	
STANDARD CONC	:	D	
STANDARD CONC	:	E	
STANDARD CONC	:	F	
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 should be allowed to reach room temperature (+20 - +25°C) prior to sample preparation. Frozen patient specimens should be brought to room temperature slowly and gently mixed by hand. Do not vigorously vortex or mix patient 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 Multipipette 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 Multipipette 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. Lyphochek⁴. 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 (13).

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.

Please also refer to the section "PROCEDURAL NOTES".

⁴ Lyphochek is a registered trademark of Bio-Rad Laboratories Inc.

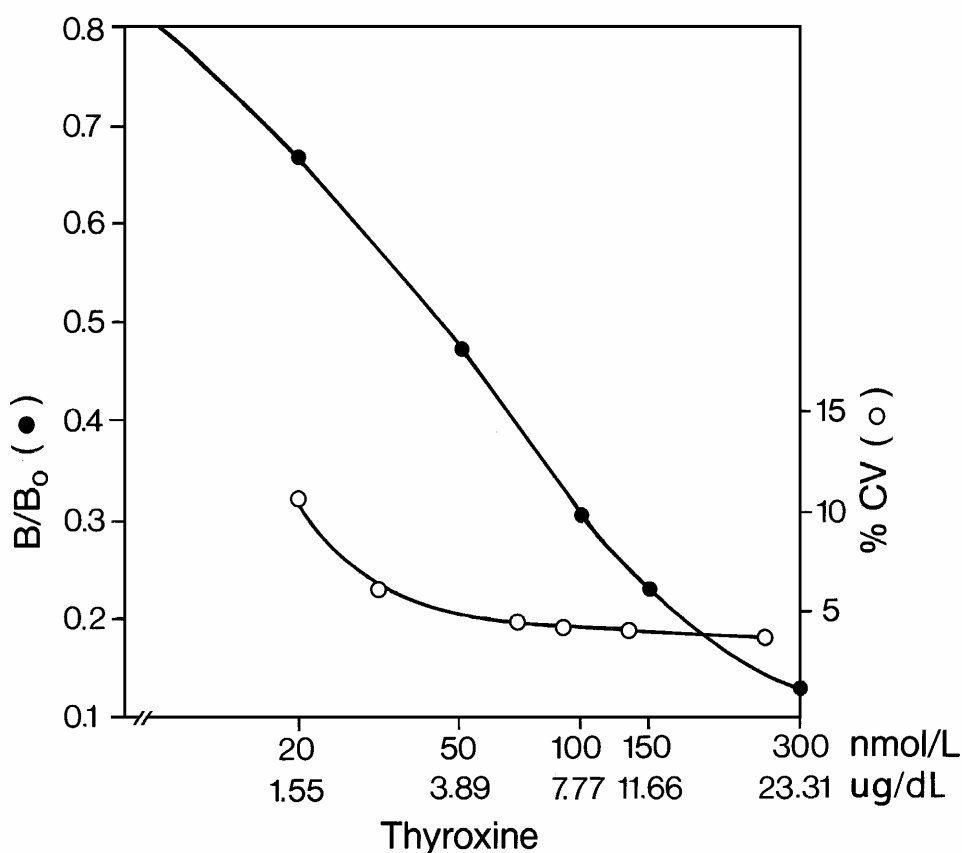
EXPECTED VALUES⁵ AND INTERPRETATION OF RESULTS

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.

Reference range, obtained from 224 healthy men and women with the DELFIA Thyroxine assay, is 69 - 141 nmol/L (5.36 - 11.0 µg/dL). The mean value is 100 nmol/L (7.77 µg/dL).

ANALYTICAL PERFORMANCE CHARACTERISTICS

A typical standard curve and precision profile (n = 10) for the DELFIA Thyroxine assay are shown below.



Precision⁶: The intra-assay (within run) precision of the DELFIA Thyroxine assay is shown below.

Serum pool	Number of duplicate determinations	Mean value		% CV
		nmol/L	µg/dL	
1	10	30	2.3	6.3
2	10	134	10.4	3.6
3	10	283	22.0	2.9

⁵ Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.

⁶ as above

The inter-assay (between run) precision of the DELFIA Thyroxine assay is shown below.

Serum pool	Number of duplicate determinations	Mean value		% CV
		nmol/L	µg/dL	
1	10	47	3.6	7.0
2	10	101	7.8	5.5
3	10	189	14.7	6.2

Analytical sensitivity⁷: The analytical sensitivity of the DELFIA Thyroxine assay is typically better than 5 nmol/L (0.39 µg/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).

Recovery⁸: Spiked serum samples were prepared by adding varying levels of thyroxine to pooled serum specimens containing a known amount of thyroxine. Recoveries were in the range of 95 - 106 % with a mean value of 101.4 % (n = 15).

Cross reactivity⁹: (at the 50 % displacement level):

Substance	Cross reactivity %
L-Thyroxine	100
D-Thyroxine	56
3,3',5-Triiodothyroacetic acid	14.5
3,3',5-L-Triiodothyronine	5.2
3,5-Diiodo-L-Tyrosine (DIT)	< 0.1
3-Iodo-L-Tyrosine (MIT)	< 0.1
3,5-Diiodo-L-Thyronine	< 0.1
5,5-Diphenylhydantoin	< 0.1

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 PerkinElmer Life and Analytical Sciences, Wallac Oy and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

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

⁷ Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.

⁸ as above

⁹ as above

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13. Westgard, J.O. et al. (1981): A multi-rule chart for quality control. *Clin. Chem.* **27**, 493-501.

PATENTS

This test system is covered by the following patents:

Europe (Austria, Belgium, Italy, Switzerland, Holland, UK, France): 0064484, 0139675

Federal Republic of Germany: P32722605-08, P3462252.7

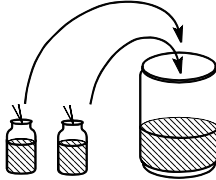
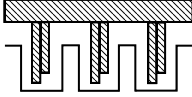
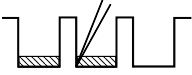
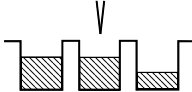

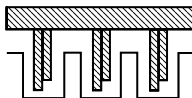
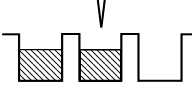
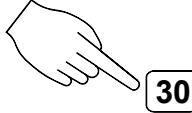
Sweden: 8102753-4

USA: 4,565,790, 4,808,541

Last revision May 2005

DELFLIA[®] T₄ kit

Summary Protocol Sheet

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