

**A042-201**

**DELFLIA<sup>®</sup>**  
**hTSH Ultra**

**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.**



**SYMBOLS**

Batch code



Packing number



Catalog number



Use by



Temperature limitation



Store in the dark



Contains sufficient for &lt;n&gt; tests



Consult instructions for use



Manufacturer



This way up



Recyclable

## DELFLIA<sup>®</sup> hTSH Ultra kit

### APPLICATION

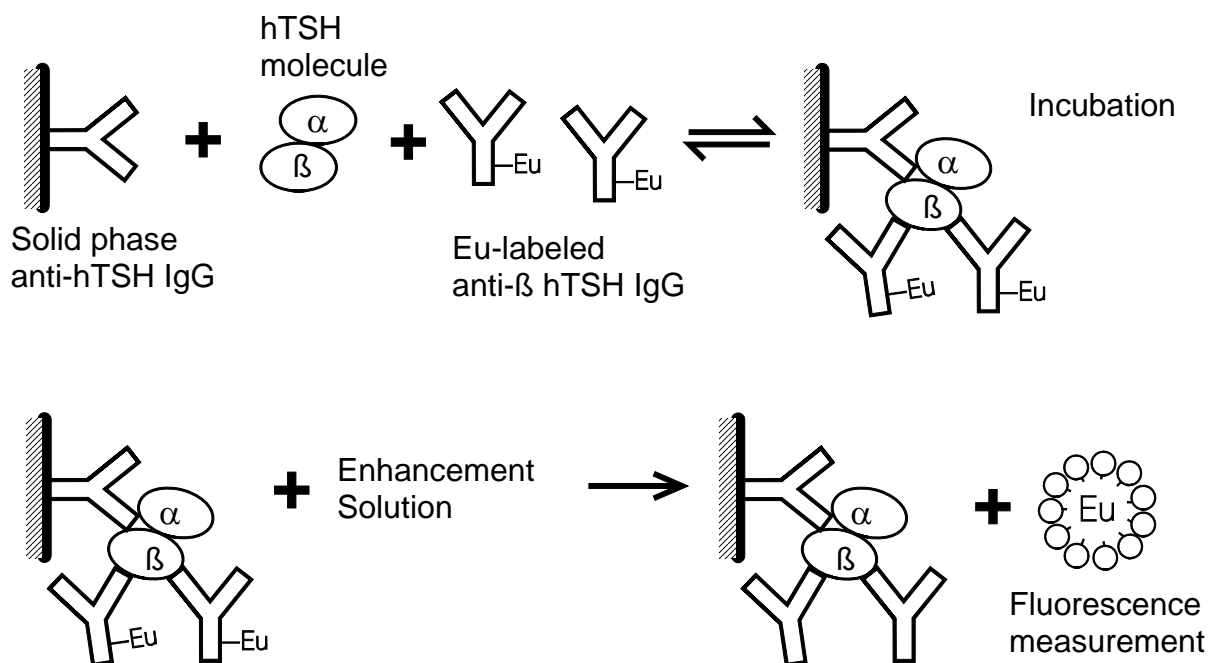
This kit is intended for the quantitative determination of human thyrotropin (hTSH) in serum.

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

### PRINCIPLES OF THE ASSAY

The DELFLIA<sup>®</sup> hTSH Ultra assay is a solid phase, two-site fluoroimmunoassay based on the direct sandwich technique in which three monoclonal antibodies (derived from mice) are directed against separate antigenic determinants on the hTSH molecule. Standard, control and samples containing hTSH are reacted simultaneously with immobilized monoclonal antibodies directed against the hTSH molecule and with europium-labeled monoclonal antibodies directed against different specific antigenic sites on the beta subunit. The complete assay requires only one incubation step.

Enhancement Solution dissociates europium ions from the labeled antibody into solution where they form highly fluorescent chelates with components of the Enhancement Solution. The fluorescence in each well is then measured. The fluorescence of each sample is proportional to the concentration of hTSH in the sample (1,2,3,4).



## KIT CONTENTS

Each DELFIA hTSH Ultra 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
hTSH Standards (approx. values)	6 vials, 1.4 mL	+2 - +8°C until expiry date stated on the vial label.
A 0 µU/mL	The exact hTSH concentrations are given on the lot specific quality control certificate included in the kit.	
B 0.03 µU/mL		
C 0.1 µU/mL		
D 1.0 µU/mL		
E 10 µU/mL		
F 100 µU/mL		

The ready-for-use standards are in Tris-HCl buffered salt solution with bovine serum albumin, and < 0.1% sodium azide as preservative. The standards have been calibrated against the Thyroid-Stimulating Hormone, Human, for Immunoassay, Third International Standard, NIBSC Code 81/565.

Anti-hTSH-Eu tracer stock solution (~ 40 µg/mL) (mouse monoclonal)	1 vial, 1.1 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.

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 II<sup>1</sup> as preservative.

hTSH Ultra Assay Buffer	1 bottle, 20 mL	+2 - +8°C 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, casein, mouse IgG, Tween 20, an inert red dye, and < 0.1% sodium azide as preservative.

<sup>1</sup> Germall is a registered trademark of ISP Investments, Inc.

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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-100<sup>2</sup>, acetic acid and chelators.

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Anti-hTSH Microtitration Strips. 8 x 12 wells coated with antibodies directed against the hTSH molecule (mouse monoclonal)	1 plate	+2 - +8°C until expiry date stated on the label.
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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 hTSH Ultra 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 hTSH Ultra Assay Buffer and the diluted tracer 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)
6. DELFIA Diluent II (prod. nos. B131-100 and B132-100)

In addition to the DELFIA system the following are required:

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

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<sup>2</sup> 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 (hemoglobin  $\leq 5$  g/L), lipemic ( $\leq 5$  g/L) and icteric (bilirubin  $\leq 500$   $\mu\text{mol/L}$ ) serum samples do not interfere with the assay.

Specimens giving hTSH values above the highest standard (100  $\mu\text{U/mL}$ ) should be diluted with the DELFIA Diluent II, and the result multiplied with the appropriate dilution factor.

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

Handle all specimens as potentially infectious. 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.

Reagents contain sodium azide ( $\text{NaN}_3$ ) 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).

Anti-hTSH-Eu tracer solution

**Prepare within one hour of use.**

Prepare the needed volume of tracer dilution by mixing 75  $\mu\text{L}$  of tracer stock solution with 1.5 mL of hTSH Ultra Assay Buffer per strip (see table in the Summary Protocol Sheet).

**It is important that the hTSH Ultra 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 working solution.

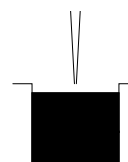
- Transfer the required number of microtitration strips to a strip frame.

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.

- Pipette 100  $\mu\text{L}$  of the hTSH 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
1 <sup>st</sup> Unk	1 <sup>st</sup> Unk	2 <sup>nd</sup> Unk	2 <sup>nd</sup> Unk	3 <sup>rd</sup> Unk	3 <sup>rd</sup> Unk	etc.						B
												C etc.

- Add 100  $\mu\text{L}$  of diluted tracer solution to each well using **the recommended Eppendorf Multipipette** 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.



- Cover the frame containing the strips and incubate for 2 hours ( $\pm$  10 minutes) at room temperature with **slow** shaking on the DELFIA Plateshake.
- After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 42 (wash).

7. Add 200  $\mu$ L 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 1234 fluorometer select kit program 42 or MultiCalc<sup>® 3</sup> protocol "42 TSHU" for automatic measurement and result calculation.

When using VICTOR<sup>2</sup> D start the measurement from the Start Wizard, select "TSHU" from Protocols/Kits panel "Thyroid" and define the number of plates and samples.

Check the parameter group for program 42 or the MultiCalc protocol "42 TSHU". 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	:	IFMA	
FITTING METHOD	:	SPLINE SMOOTHED	
X-AXIS	:	LOGARITHMIC	
Y-AXIS	:	LOGARITHMIC	
BLANKS	:	2	
STANDARDS	:	5	
STANDARD REPLICATES	:	2	
STANDARD CONC	:	B	<b>(Make sure that the hTSH standard concentrations correspond to those given on the lot specific quality control certificate. If this is not the case, enter the new concentrations.)</b>
STANDARD CONC	:	C	
STANDARD CONC	:	D	
STANDARD CONC	:	E	
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.

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<sup>3</sup> MultiCalc is a registered trademark of PerkinElmer, Inc.  
VICTOR is a trademark of PerkinElmer, Inc.



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.
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).

### **Calibration**

hTSH Standards in the range of 0 - 100  $\mu\text{U}/\text{mL}$  are provided with the kit. These have been calibrated against the Thyroid-Stimulating Hormone, Human, for Immunoassay, Third International Standard, NIBSC Code 81/565. The standards should be run in duplicate on each plate.

## **LIMITATIONS OF THE PROCEDURE**

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

Specimens giving hTSH values above the highest standard (100  $\mu\text{U}/\text{mL}$ ) should be diluted with the DELFIA Diluent II, and the result multiplied with the appropriate dilution factor.

Please also refer to the section "PROCEDURAL NOTES".

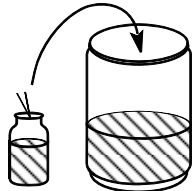
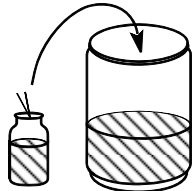

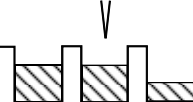

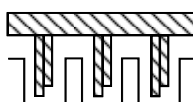
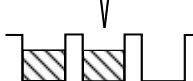
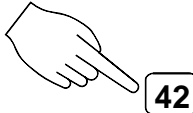
**REFERENCES**

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2. 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.
3. Lövgren, T., Hemmilä, I., Pettersson, K. and Halonen, P. (1985): Time-resolved fluorometry in immunoassays. In *Alternative Immunoassays*. Ed. W.P. Collins. John Wiley & Sons Ltd., England, pp. 203-217.
4. Lövgren, T., Hemmilä, I., Petterson, K., Eskola, J.U. and Bertoft, E. (1984): Determination of hormones by time-resolved fluoroimmunoassay. *Talanta* **31**, 909-916.

March 28, 2012

# DELFLIA<sup>®</sup> hTSH Ultra kit

## Summary Protocol Sheet

		Strips	Tracer stock solution (µL)	Buffer (mL)
		1	75	1.5
		2	150	3.0
		3	225	4.5
		4	300	6.0
		5	375	7.5
		6	450	9.0
		7	525	10.5
		8	600	12.0
Dilute tracer (see table)				
Add standards and unknowns		100 µL		
Add tracer dilution		100 µL		
Incubate		2 h (± 10 min.) slow shaking at RT		
Wash		Program 42 (x 6)		
Enhance		200 µL, 5 min. slow shaking		
Count		KIT 42 (check concentrations from QC certificate)		