

A031-101

DELFLIA[®]
hLH Spec

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



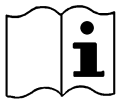
Temperature limitation



Store in the dark



Contains sufficient for <n> tests



Consult instructions for use



Manufacturer



This way up



Recyclable

DELFIA[®] hLH Spec kit

APPLICATION

This kit is for the quantitative determination of human luteinizing hormone (hLH, luteotropin) in serum.

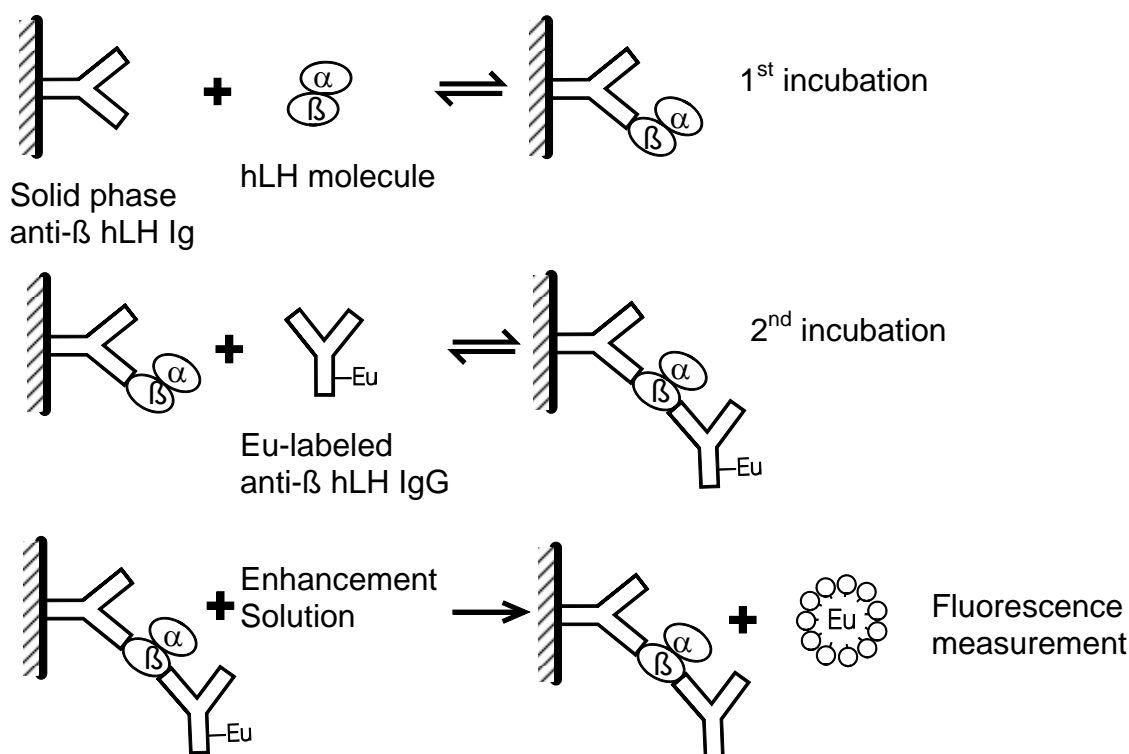
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PRINCIPLES OF THE ASSAY

The DELFIA[®] hLH Spec assay is a solid phase, two-site fluoroimmuno-metric assay based on the direct sandwich technique, in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the hLH molecule. Standard, control and samples containing hLH are first reacted with immobilized monoclonal antibodies directed against a specific antigenic site on the hLH β -subunit. Europium-labeled antibodies directed against another specific antigenic site on the hLH β -subunit are then reacted with the intact hLH or its subunit already bound to the solid-phase antibody.

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 is proportional to the concentration of hLH in the sample (1,2,3).

It is also possible to run a rapid assay using only three standards and shorter incubation times.



KIT CONTENTS

Each DELFIA hLH Spec 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
hLH Standards (approx. values)	6 vials, 1.0 mL	+2 - +8°C until expiry date stated on the vial label.
A 0 U/L		The exact hLH concentrations are given on the lot specific quality control certificate included in the kit.
B 0.6 U/L		
C 3 U/L		
D 15 U/L		
E 75 U/L		
F 250 U/L		

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 WHO 2nd International Standard for pituitary LH for immunoassay (coded 80/552).

Anti-hLH-Eu tracer stock solution (~20 µg/mL) (mouse monoclonal)	1 vial, 0.65 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 II¹ as preservative.

hLH Spec Assay Buffer	1 bottle, 50 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, Tween 40, blockers, hCG-blocking reagent, 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.
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Ready-for-use Enhancement Solution with Triton² X-100, acetic acid and chelators.

Anti-hLH Microtitration Strips. 8 x 12 wells coated with antibodies against a specific site on the β-subunit of the hLH 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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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 hLH Spec 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 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)

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. Plasma samples can also be used. Hemolytic (hemoglobin ≤ 10 g/L), lipemic (triglycerides ≤ 10 g/L) or icteric (bilirubin ≤ 340 μ mol/L) samples do not interfere.

Serum and plasma samples can be stored 6 days at +2 - +8°C. For longer periods store samples at -20°C. Repeated freezing and thawing should be avoided.

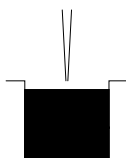
WARNINGS AND PRECAUTIONS

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 (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.

4. Pipette 200 μ L of hLH Spec Assay Buffer 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 1 h (\pm 10 minutes) at room temperature with **slow** shaking using the DELFIA Plateshake.
6. After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 31 (wash 1).
7. Add 200 μ L of diluted Anti-hLH-Eu tracer solution to each well using **the recommended Eppendorf Multipette** after discarding the first aliquot, or use the DELFIA Dispense Unit. Pipetting should be as for the assay buffer in step 4 above.
8. Incubate the frame for 15 minutes (\pm 5 minutes) with **slow** shaking at room temperature.
9. After the second incubation step, aspirate and wash wash each strip with the DELFIA Platewash using program 31 (wash 2).
10. Add 200 μ L 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.
11. 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.
12. 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 31 or MultiCalc[®] ³ protocol "31 LHSP" for automatic measurement and result calculation.

When using VICTOR² D start the measurement from the Start Wizard, select "LHSP" from Protocols/Kits panel "Fertility" and define the number of plates and samples.

Check the parameter group for program 31 or the MultiCalc protocol "31 LHSP". 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	:	IFMA	
FITTING METHOD	:	SPLINE SMOOTHED	
X-AXIS	:	LOGARITHMIC	
Y-AXIS	:	LOGARITHMIC	
BLANKS	:	2	
STANDARDS	:	5	
STANDARD REPLICATES	:	2	
STANDARD CONC	:	B	(Make sure that the hLH 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	
UNKNOWN REPLICATES	:	2	

RAPID ASSAY PROCEDURE

Use the standards A, C and E to draw the standard curve, and shorten the two incubation periods each to 15 minutes. Otherwise the assay procedure is exactly as described above.

In order that results of the rapid assay can be meaningfully compared, it is important to limit the number of test specimens so that pipetting can be done within 5 minutes for each pipetting cycle. This will keep variations in the reaction (resulting from the incomplete immunoreaction due to the short incubation times) within acceptable limits.

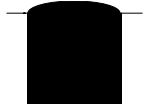
Create a new parameter group for automatic calculation of results as follows:

ASSAY TYPE	:	IFMA	
FITTING METHOD	:	SPLINE SMOOTHED	
X-AXIS	:	LOGARITHMIC	
Y-AXIS	:	LOGARITHMIC	
BLANKS	:	2	
STANDARDS	:	2	(Make sure that the hLH standard concentrations correspond to those given on the lot specific quality control certificate. If this is not the case, enter the new concentrations.)
STANDARD REPLICATES	:	2	
STANDARD CONC	:	C	
STANDARD CONC	:	E	
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.

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.

Due to high levels of hCG during pregnancy, the DELFIA hLH Spec kit is not recommended for measuring hLH concentrations during pregnancy or immediately post-partum.

Please also refer to the section "PROCEDURAL NOTES".




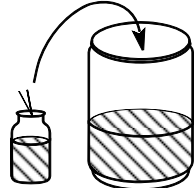
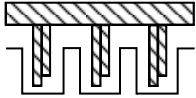

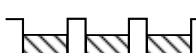
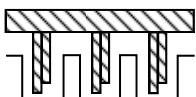

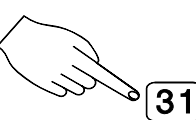
REFERENCES

1. Soini, E. and Kojola, H. (1983): Time-resolved fluorometer for lanthanide chelates - a new generation of nonisotopic immunoassays. *Clin. Chem.* **29**, 65-68.
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., Petterson, K., Eskola, J.U. and Bertoft, E. (1984): Determination of hormones by time-resolved fluoroimmunoassay. *Talanta* **31**, 909-916.

February 23, 2012

DELFLIA[®] hLH Spec kit

Summary Protocol Sheet

Add standards and unknowns		25 µL		
Add buffer		200 µL		
Incubate		1 h slow shaking at RT or 15 min. slow shaking at RT (rapid prot.)		
Dilute tracer (see table)		Strips		
		Tracer stock solution (µL)		
		Buffer (mL)		
		1	20	3
		2	40	6
		3	60	9
		4	80	12
		5	100	15
		6	120	18
7	140	21		
8	160	24		
Wash		Program 31 (x 1)		
Add tracer dilution		200 µL		
Incubate		15 min. slow shaking at RT		
Wash		Program 31 (x 6)		
Enhance		200 µL, 5 min. slow shaking		
Count		KIT 31 (check concentrations from QC certificate)		