

DELFLIA[®] hFSH Reagents R017-201

For Scientific Research Use Only.

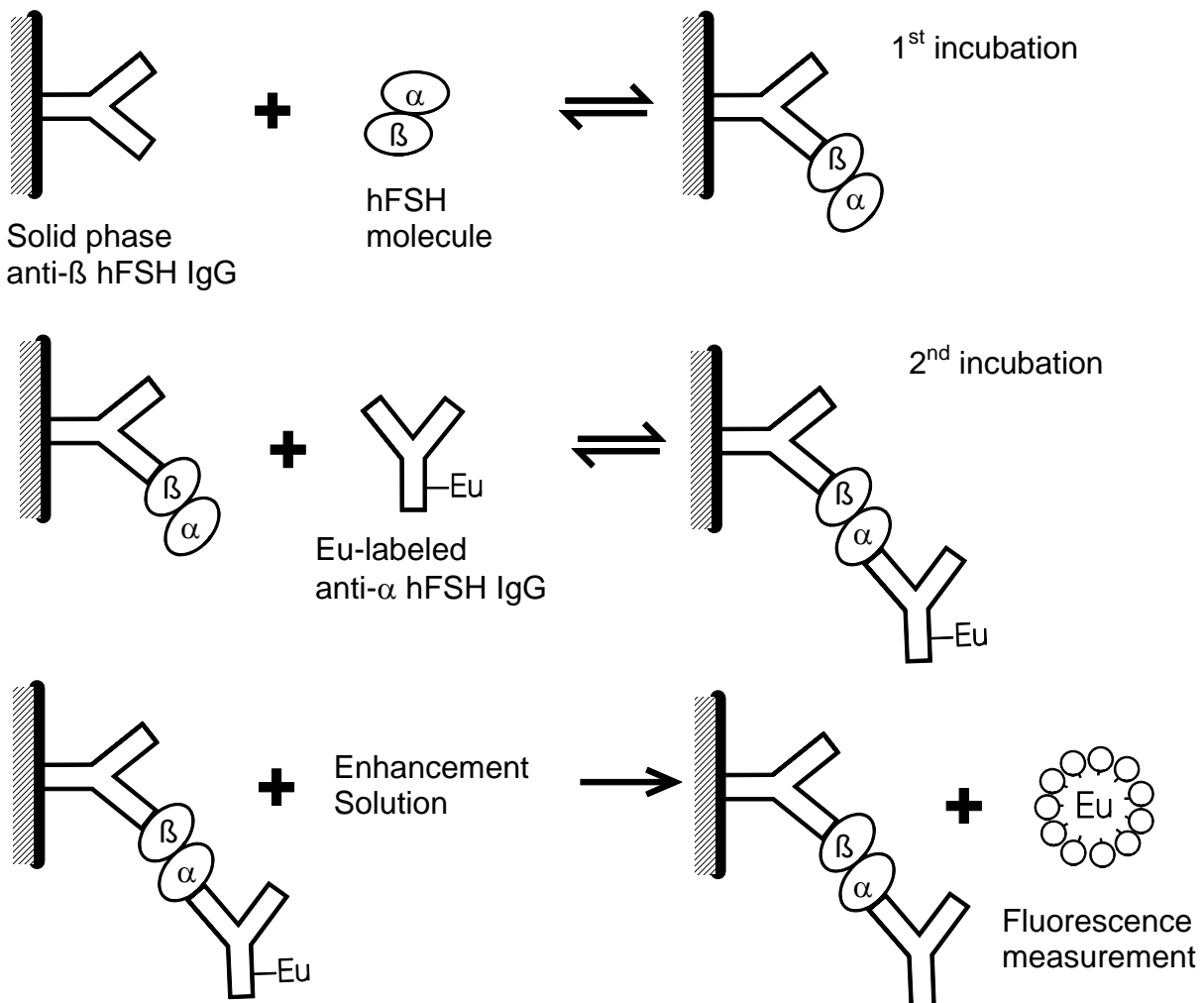
This product is not to be used for *In Vitro* or *In Vivo* Diagnosis.

PRINCIPLES OF THE ASSAY

This product has been developed for the quantitative determination of human follicle stimulating hormone (hFSH) in serum.

The DELFLIA[®] hFSH assay is a solid phase, two-site fluoroimmunoassay in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the hFSH molecule. Standard, control and samples containing hFSH are first reacted with immobilised monoclonal antibodies directed against a specific antigenic site on the β -subunit of hFSH. Europium-labeled antibodies directed against a specific antigenic site on the α -subunit of hFSH are then reacted with the hFSH molecule, 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 quantity of hFSH in the sample (1,2).



PACKAGE CONTENTS

Each DELFIA hFSH package contains reagents for 96 assays.

The expiry date of the complete package 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
hFSH Standards (approx. values)	6 vials, 1.1 mL	+2 - +8°C until expiry date stated on the vial label.
A 0 U/L	The exact hFSH concentrations are given on the lot specific quality control certificate included in the package.	
B 1 U/L		
C 4 U/L		
D 16 U/L		
E 64 U/L		
F 256 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 2nd IRP of Pituitary FSH/LH (ICSH) human for bioassay (78/549).

Anti-hFSH-Eu tracer stock solution (~ 20 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 tracer is in Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, mouse IgG, 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.

¹ Germall is a registered trademark of Sutton Laboratories Inc.

Multibuffer	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, mouse IgG, diethylenetriaminepentaacetic acid, Tween 40, blockers, 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 Triton X-100², acetic acid and chelators.

Anti-hFSH Microtitration Strips. 8 x 12 wells coated with antibodies against the β -subunit of the hFSH 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 PACKAGE

The DELFIA hFSH reagents are part of a complete system of 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 Multibuffer and the diluted tracer 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)

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

In addition to the DELFIA system the following are required:

- precision pipettes for dispensing microliter volumes
- pipettes for dispensing the milliliter volumes of Multibuffer required to prepare the tracer dilution
- distilled water

COLLECTION AND HANDLING OF SERUM AND PLASMA SAMPLES

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Plasma samples can also be used. Hemolytic, lipemic or icteric serum samples do not interfere.

Samples can be stored for 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 scientific research use only. This product is not to be used for *in vitro* or *in vivo* diagnosis.

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.

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).

Anti-hFSH-Eu tracer

Prepare within one hour of use.

Prepare the needed volume of tracer dilution by mixing 30 μL tracer stock solution with 3.0 mL of Multibuffer per strip (see table in the Summary Protocol Sheet).

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

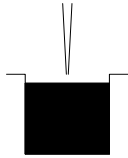
We advise the use of a disposable plastic container to prepare the tracer working solution.

- 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 25 μL of the hFSH Standards (Std) and serum samples (unknowns - Unk) into the strip wells according to the following table.

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 Multibuffer 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 3 hours (\pm 10 minutes) with **slow** shaking at room temperature using the DELFIA Plateshake.
- After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 17 (wash 1).
- Add 200 μL of diluted Anti-hFSH-Eu tracer solution to each well using **the recommended Eppendorf Multipette**. Pipetting should be as for the Multibuffer in step 4 above.

8. Incubate the frame for 30 minutes (\pm 10 minutes) at room temperature **without shaking**.
9. After the second incubation step, aspirate and wash each strip with the DELFIA Platewash using program 17 (wash 2).
10. Add 200 μ 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.
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 program 17 or MultiCalc^{® 3} protocol "17 FSH" for automatic measurement and result calculation.

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

Check the parameter group for program 17 or the MultiCalc protocol "17 FSH", and correct it, if it differs from the following (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	
STANDARD CONC	:	C	(Make sure that the hFSH 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	:	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 product. The reagents supplied with this package are intended for use as an integral

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

unit. Do not mix identical reagents from packages having different lot numbers. Do not use reagents after the expiry date printed on the package label.

2. Reagents should be allowed to reach room temperature (+20 - +25°C) prior to sample preparation. Frozen samples should be brought to room temperature slowly and gently mixed by hand. Do not vigorously vortex or mix samples.
3. 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.

4. 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.

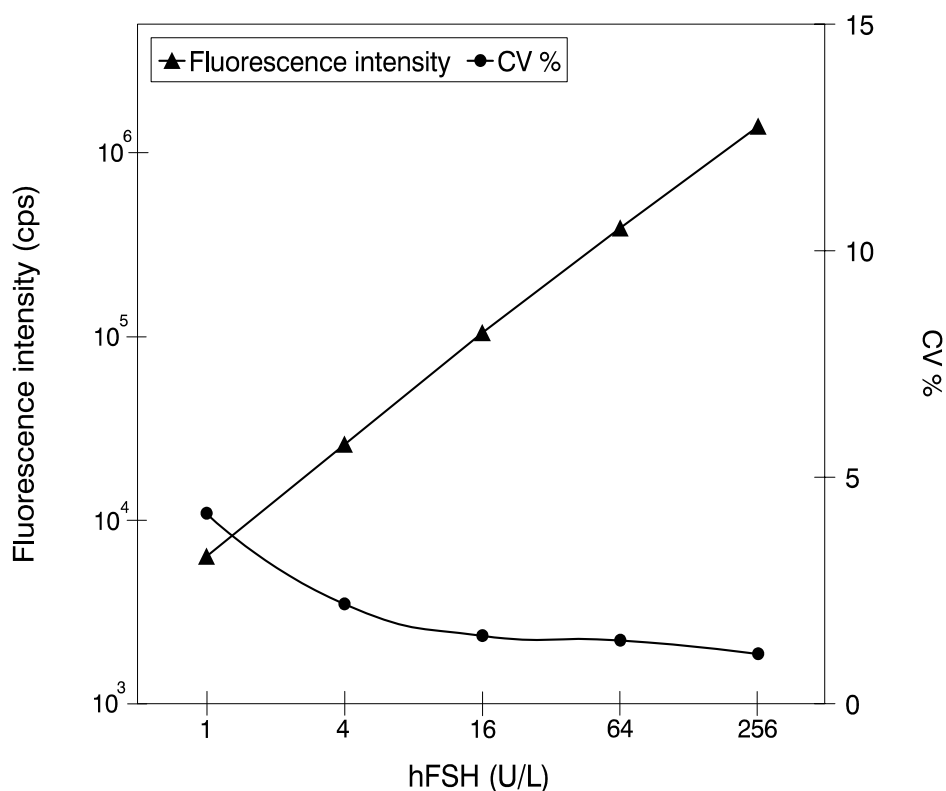
When using the DELFIA Plate Dispense and DELFIA Dispense Unit, please refer to the manual.

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).

ANALYTICAL PERFORMANCE CHARACTERISTICS

A typical standard curve and a precision profile obtained with the DELFIA hFSH assay are shown below. The precision profile was calculated from 301 duplicate measurements of serum samples using the MultiCalc data management program.



Precision⁴: The variation of the DELFIA hFSH assay was determined in 27 assays with 2 replicates, and the analysis of variance approach was used to calculate the following variations:

Serum sample	Total mean value (U/L)	Intra-assay variation (% CV)	Inter-assay variation (% CV)	Total variation (% CV)
1	2.58	2.0	1.8	2.7
2	11.5	2.8	2.0	3.4
3	44.8	2.2	1.8	2.8

Analytical sensitivity⁵: The analytical sensitivity of the DELFIA hFSH assay is typically better than 0.05 U/L, if the analytical sensitivity is defined as the value which is 2 standard deviations above the mean of the zero standard measurement values (mean value + 2 SD) (n = 96).

Recovery⁶: Spiked serum samples were prepared by adding varying levels of concentrated hFSH solution to pooled serum samples containing a known amount of hFSH. Recoveries were in the range of 83 - 97 % with a mean value of 91 % (n = 6).

⁴ Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.

⁵ as above

⁶ as above

Dilution⁷: Five different serum samples in the range of 5.49 - 91.2 U/L were diluted serially with DELFIA Diluent II, and observed vs. expected hFSH concentrations were determined in 6 different dilutions. The mean results of each dilution series were in the range of 101 - 105 %, with an overall mean value of 103 % (n = 5).

Cross reactivity⁸: The cross reactivity of the DELFIA hFSH reagents with other hormones is presented in the following table:

Hormone	Added concentration	Measured apparent hFSH concentration
hTSH	324 µU/mL	< 0.7 U/L
hLH	250 U/L	< 0.2 U/L
hCG	10 ⁶ U/L	< 0.3 U/L

Hook effect⁹: hFSH concentrations were measured up to 2260 U/L; no hook effect was found.

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

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., Mikkala, 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., Eskola, J.U. and Bertoft, E. (1984): Determination of hormones by time-resolved fluoroimmunoassay. *Talanta*, **31**, 909-916.

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

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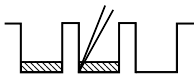
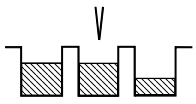
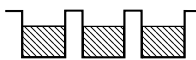
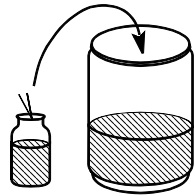
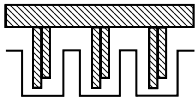
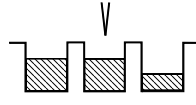

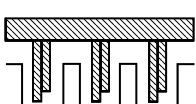
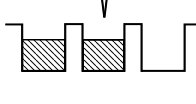
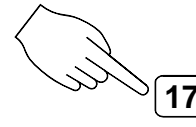
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DELFLIA[®] hFSH Reagents

Summary Protocol Sheet

Add standards and unknowns		25 μ L																											
Add buffer		200 μ L																											
Incubate		3 h slow shaking at RT																											
Dilute tracer (see table)		<table border="1"> <thead> <tr> <th>Strips</th> <th>Tracer stock solution (μL)</th> <th>Buffer (mL)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>30</td> <td>3</td> </tr> <tr> <td>2</td> <td>60</td> <td>6</td> </tr> <tr> <td>3</td> <td>90</td> <td>9</td> </tr> <tr> <td>4</td> <td>120</td> <td>12</td> </tr> <tr> <td>5</td> <td>150</td> <td>15</td> </tr> <tr> <td>6</td> <td>180</td> <td>18</td> </tr> <tr> <td>7</td> <td>210</td> <td>21</td> </tr> <tr> <td>8</td> <td>240</td> <td>24</td> </tr> </tbody> </table>	Strips	Tracer stock solution (μ L)	Buffer (mL)	1	30	3	2	60	6	3	90	9	4	120	12	5	150	15	6	180	18	7	210	21	8	240	24
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Wash		Program 17 (x 2)																											
Add tracer dilution		200 μ L																											
Incubate		30 min. without shaking at RT																											
Wash		Program 17 (x 6)																											
Enhance		200 μ L, 5 min. slow shaking																											
Count		KIT 17 (check concentrations from QC certificate)																											