

A007-101

DELFINA[®]

hCG

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

DELFLIA[®] hCG kit

APPLICATION

This kit is for the quantitative determination of human chorionic gonadotropin (hCG) in serum.

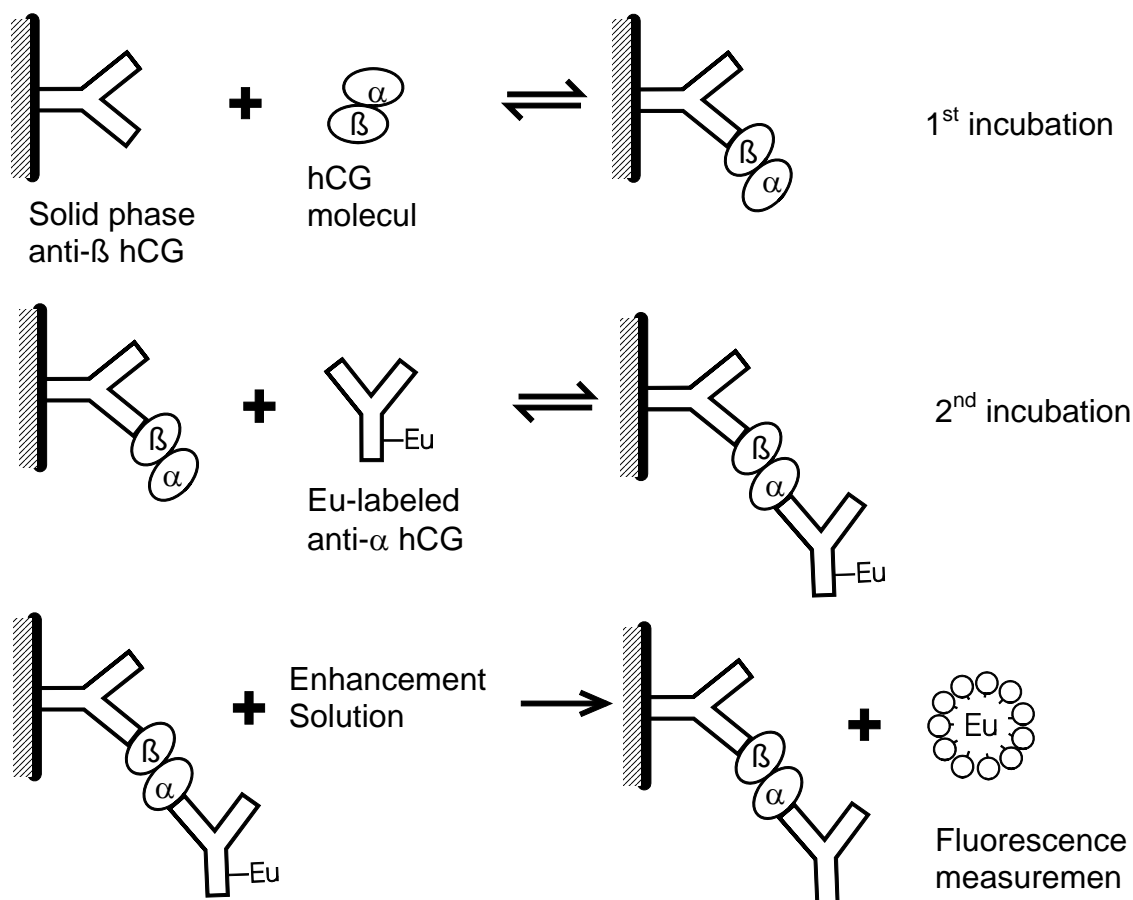
For research use only. Not for use in diagnostic procedures.

PRINCIPLES OF THE ASSAY

The DELFLIA[®] hCG 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 hCG molecule. Standard, control and samples containing hCG are first reacted with immobilized monoclonal antibodies directed against a specific antigenic site on the β subunit of hCG. Europium-labeled antibodies directed against a specific antigenic site on the α subunit are then reacted with the intact hCG 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 hCG in the sample (1,2,3,4).

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



KIT CONTENTS

Each DELFIA hCG kit contains reagents for 96 assays.

The expiry date of the unopened 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
hCG Standards (approx. value)	7 vials, 1.0 mL	+2 - +8°C until expiry date stated on the vial label.
A 0 U/L		
B 2 U/L		
C 10 U/L		
D 100 U/L		
E 1000 U/L		
F 5000 U/L		
G 10000 U/L		
	The exact hCG concentrations are given on the lot specific quality control certificate included in the kit.	

The ready-for-use standards are in normal human male serum with < 0.1 % sodium azide as preservative. The standards have been calibrated against the 4th International Standard for Chorionic Gonadotropin (75/589).

Anti-hCG-Eu tracer stock solution (~ 20 µg/mL) (mouse monoclonal)	1 vial, 0.95 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. Germall II¹ added as preservative.

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

DELFLIA 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, mouse IgG, Tween 40, 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-hCG Microtitration Strips. 8 x 12 wells coated with antibodies against the β subunit of the hCG 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 DELFLIA hCG kit is part of a complete system of reagents and instrumentation. The DELFLIA 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 - DELFLIA Platewash (prod. no. 1296-026)
3. Automatic shaker - DELFLIA Plateshake (prod. no. 1296-003/004)
4. Pipette for dispensing DELFLIA Buffer and the diluted tracer solution - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively DELFLIA Plate Dispense with the DELFLIA 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 DELFLIA Plate Dispense (prod. no. 1296-041)
6. DELFLIA Diluent I (prod. nos. B127-100 and B128-100) or DELFLIA Diluent II (prod. nos. B131-100 and B132-100)

In addition to the DELFLIA system the following are required:

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

- 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 specimens can also be used. Hemolytic, lipemic or icteric samples do not interfere with the assay.

If the hCG concentrations in specimens exceed (or are expected to exceed) 5000 U/L, they should be diluted 100-fold with DELFIA Diluent I or Diluent II to bring them below 5000 U/L.

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 to prevent hCG denaturation (10).

WARNINGS AND PRECAUTIONS

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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 derivative should be observed. Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88-8395 on laboratory safety procedures or any other local or national regulation.

Handle all 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 deionized water to give a buffered wash solution (pH 7.8).

Anti-hCG-Eu tracer

Prepare within one hour of use.

Prepare the needed volume of tracer dilution by mixing 60 µL of tracer stock solution with 3.0 mL of DELFIA Buffer per strip (see table in the Summary Protocol Sheet).

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

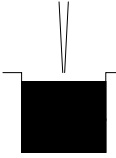
We advise the use of a disposable plastic container to prepare the tracer working solution.

- 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 25 µL of the hCG Standards (Std) and specimens (unknowns - Unk) in duplicate 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
Std G	Std G	1st Unk	1st Unk	2nd Unk	2nd Unk	3rd Unk	3rd Unk	etc.				B
												C etc.

- Add 200 µL of DELFIA 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. 
- Incubate the frame for 1 hour (\pm 10 minutes) at room temperature with **slow** shaking using the DELFIA Plateshake.
- After the first incubation step, aspirate and wash each strip with the DELFIA Platewash using program 7 (wash 1).
- Add 200 µL of diluted Anti-hCG-Eu tracer solution to each well. Pipetting should be as for the DELFIA Buffer in step 4 above.
- Incubate the frame for 30 minutes (\pm 10 minutes) with **slow** shaking at room temperature.

9. After the second incubation step, aspirate and wash each strip with the DELFIA Platewash using program 7 (wash 2).
10. 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.
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 7 or MultiCalc^{® 3} protocol "7 HCG" for automatic measurement and result calculation.

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

Check the parameter group for program 7 or the MultiCalc protocol "7 HCG". 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	:	6	
STANDARD REPLICATES	:	2	
STANDARD CONC	:	B	
STANDARD CONC	:	C	(Make sure that the hCG 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	
STANDARD CONC	:	G	
UNKNOWN REPLICATES	:	2	

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

Rapid assay procedure

When only a small number of samples need to be assayed a rapid assay protocol can be followed. Use only the 0 and 1000 U/L Standards for the standard curve. Shorten the two incubation periods to 30 and 15 minutes, respectively. 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 specimens so that pipetting can be done in 5 minutes or less for each pipetting cycle. This will keep variations in the reaction (resulting from incomplete immunoreactions due to the short incubation times) within acceptable limits.

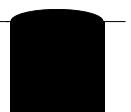
Create a new parameter group for automatic measurement and result calculation as follows:

ASSAY TYPE	:	IFMA	
FITTING METHOD	:	SPLINE SMOOTHED	
X-AXIS	:	LINEAR	
Y-AXIS	:	LINEAR	
BLANKS	:	0	
STANDARDS	:	2	(Make sure that the hCG 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	:	A	
STANDARD CONC	:	E	
UNKNOWN REPLICATES	:	2	

hCG values of the rapid assay procedure (incubation time: 30 + 15 minutes) are equivalent to concentrations obtained with the normal assay procedure (incubation time: 1 h + 30 minutes).

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 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).

LIMITATIONS OF THE PROCEDURE

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

If the hCG concentrations in specimens exceed (or are expected to exceed) 5000 U/L, they should be diluted 100-fold with DELFIA Diluent I or Diluent II to bring them below 5000 U/L.

Please also refer to the section "PROCEDURAL NOTES" and "SPECIMEN COLLECTION AND HANDLING".

REFERENCES

1. Soini, E. and Kojola, H. (1983): Time-resolved fluorometer for lanthanide chelates - a new generation of non-isotopic 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. Pettersson, K., Siitari, H., Hemmilä, I., Soini, E., Lövgren, T., Hänninen, V., Tanner, P. and Stenman, U-H. (1983): Time-resolved fluoroimmunoassay of human choriogonadotrophin. *Clin. Chem.* **29**, 60-64.
4. 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. 203-219.

February 23, 2012

DELFLIA[®] hCG kit

Summary Protocol Sheet

Add standards and unknowns		25 µL																											
Add buffer		200 µL																											
Incubate		1 h slow shaking at RT or 30 min. slow shaking at RT (rapid protocol)																											
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>60</td><td>3</td></tr> <tr><td>2</td><td>120</td><td>6</td></tr> <tr><td>3</td><td>180</td><td>9</td></tr> <tr><td>4</td><td>240</td><td>12</td></tr> <tr><td>5</td><td>300</td><td>15</td></tr> <tr><td>6</td><td>360</td><td>18</td></tr> <tr><td>7</td><td>420</td><td>21</td></tr> <tr><td>8</td><td>480</td><td>24</td></tr> </tbody> </table>	Strips	Tracer stock solution (µL)	Buffer (mL)	1	60	3	2	120	6	3	180	9	4	240	12	5	300	15	6	360	18	7	420	21	8	480	24
		Strips	Tracer stock solution (µL)	Buffer (mL)																									
		1	60	3																									
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		5	300	15																									
		6	360	18																									
		7	420	21																									
8	480	24																											
Wash		Program 7 (x 2)																											
Add tracer dilution		200 µL																											
Incubate		30 min. slow shaking at RT or 15 min. slow shaking at RT (rapid protocol)																											
Wash		Program 7 (x 6)																											
Enhance		200 µL, 5 min. slow shaking																											
Count		KIT 7 (check concentrations from QC certificate)																											