

DELFLIA[®] Prolactin Reagents R018-301

For Scientific Research Use Only.

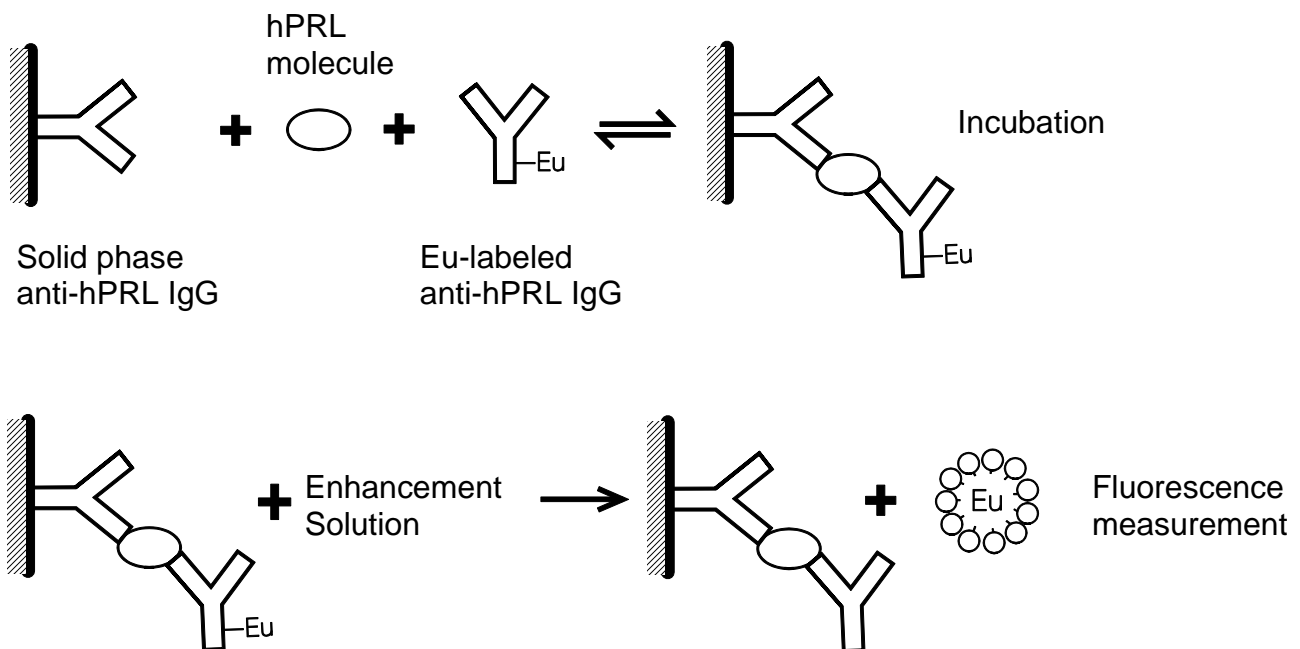
This product is not to be used for *In Vitro* or *In Vivo* Diagnosis.

PRINCIPLES OF THE ASSAY

This product has been developed for the quantitative determination of human prolactin (hPRL) in serum.

The DELFLIA[®] Prolactin assay is a solid phase, two-site fluoroimmunoassay based on the direct sandwich technique in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the hPRL molecule. Standards, control and samples containing hPRL are reacted simultaneously with immobilized monoclonal antibodies directed against a specific antigenic site on the hPRL molecule and europium-labeled antibodies directed against a different antigenic site.

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 from each sample is proportional to the concentration of hPRL in the sample (1,2).



PACKAGE CONTENTS

Each DELFIA Prolactin package 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 package components are stable for up to 2 weeks when used as described in the section "ASSAY PROCEDURE".

Reagents

Component	Quantity	Shelf life and storage
Prolactin Standards (approx. values)	6 vials, 1.1 mL	+2 - +8°C until expiry date stated on the vial label.
A 0 ng/mL 0 mU/L	The exact Prolactin concentrations are given on the lot specific quality control certificate included in the package.	
B 0.25 ng/mL 9 mU/L		
C 2.5 ng/mL 90 mU/L		
D 25 ng/mL 900 mU/L		
E 125 ng/mL 4500 mU/L		
F 250 ng/mL 9000 mU/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 3rd International Standard for Prolactin (coded 84/500).

Conversion factor: 1 ng/mL = 36 mU/L.

Anti-Prolactin-Eu tracer stock solution (~ 20 µg/mL) (mouse monoclonal)	1 vial, 0.8 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.

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-Prolactin Microtitration Strips. 8 x 12 wells coated with antibodies against hPRL (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 Prolactin 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 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)

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

The DELFIA Prolactin assay is intended for use with serum samples. Collect blood by venipuncture, and separate the serum or plasma by centrifugation. Heparin plasma can also be used, but plasma containing EDTA or citrate cannot be used due to chelating effects on europium. Hemolytic, lipemic and icteric samples do not interfere with the assay.

Plasma and serum samples can be stored 2 days at +2 - +8°C. For longer periods store serum samples at -20°C. Repeated freezing and thawing should be avoided. Do not freeze plasma samples.

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.

Please note that in some prolactinoma cases the prolactin concentration can be very high and the hook effect should be considered.

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.
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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-Prolactin-Eu tracer

Prepare within one hour of use.

Prepare the needed volume of tracer dilution by mixing 40 μL tracer stock solution with 3 mL of Multibuffer per strip (see table in the Summary Protocol Sheet).

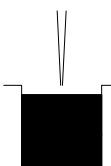
It is important that the Multibuffer does not come into contact with tracer stock solution not intended for immediate use.

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.

- Add 200 μL of diluted Anti-Prolactin-Eu tracer solution to each well using **the recommended Eppendorf Multipette** after discarding the first aliquot, or use the DELFIA Dispense Unit.



- Pipette 25 μL of the Prolactin Standards (Std) and 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.

- Incubate the frame for 90 minutes (\pm 10 minutes) at room temperature with **slow** shaking using the DELFIA Plateshake.
- After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 18 (1 Inc, 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 program 18 or MultiCalc^{® 3} protocol "18 PRL" for automatic measurement and result calculation.

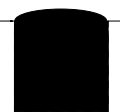
When using VICTOR² D start the measurement from the Start Wizard, select "PRL" from Protocols/Kits panel "Fertility" and define the number of plates and samples.

Check the parameter group for program 18 or the MultiCalc protocol "18 PRL", 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	(Make sure that the Prolactin 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	

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 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.



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

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 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.

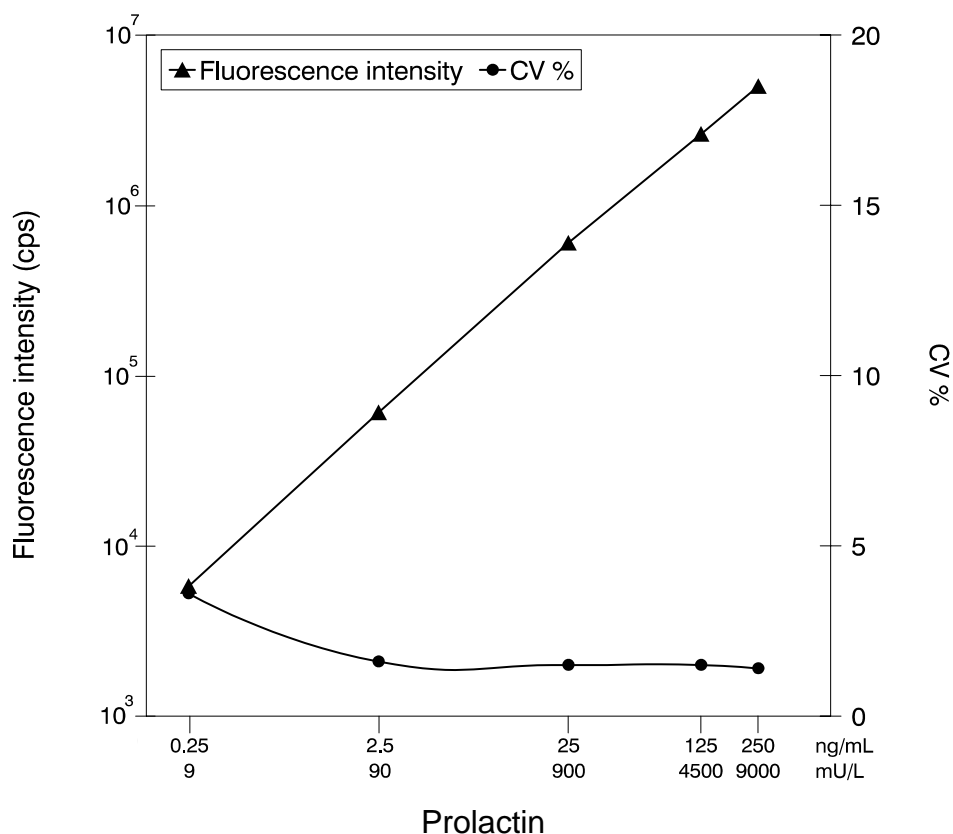
When using the DELFIA Plate Dispense and DELFIA Dispense Unit, please refer to the manual.

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).

ANALYTICAL PERFORMANCE CHARACTERISTICS

A typical standard curve and a precision profile obtained with the DELFIA Prolactin reagents are shown below. The precision profile was calculated from 400 duplicate measurement of standards and serum samples using the MultiCalc data management program.



Precision⁴: The variation of the DELFIA Prolactin 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		Intra-assay variation (% CV)	Inter-assay variation (% CV)	Total variation (% CV)
	ng/mL	mU/L			
1	3.1	113	2.0	1.8	2.7
2	56.2	2023	1.9	3.4	3.8
3	109	3924	1.4	3.4	3.7

Analytical sensitivity⁵: The analytical sensitivity of the DELFIA Prolactin reagents is typically better than 0.04 ng/mL (1.44 mU/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 hPRL to pooled serum samples containing a known amount of hPRL. Recoveries were in the range of 85 - 101 % with a mean value of 96 % (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 13.2 - 110 ng/mL (475 - 3960 mU/L) hPRL were diluted serially with DELFIA Diluent II, and observed vs. expected prolactin concentrations were determined in 6 different dilutions. The mean results of each dilution series were in the range of 97 - 108 %, with an overall mean value of 103 % (n = 5).

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

Hormone	Added concentration	Measured apparent hPRL concentration
hLH	500 U/L	non-detectable
hCG	170000 U/L	non-detectable
hFSH	20000 U/L	non-detectable
hTSH	10000 U/L	non-detectable
hPL	500 µg/mL	4.2 ng/mL
hGH	50 µg/mL	1.9 ng/mL

Hook effect⁹: There is no hook effect up to hPRL concentration 2500 ng/mL (90,000 mU/L).

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

Last revision October 2004

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Manufactured by:

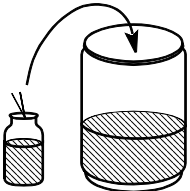
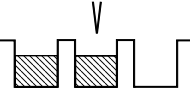
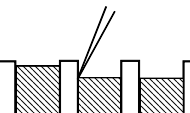
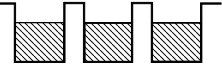
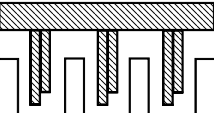
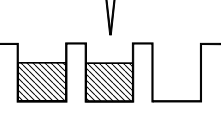
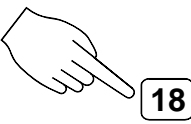
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DELFI[®] Prolactin Reagents

Summary Protocol Sheet

Dilute tracer (see table)		Strips	Tracer stock solution (µL)	Buffer (mL)
		1	40	3
		2	80	6
		3	120	9
		4	160	12
		5	200	15
		6	240	18
		7	280	21
		8	320	24
Add tracer dilution		200 µL		
Add standards and unknowns		25 µL		
Incubate		90 min. slow shaking at RT		
Wash		Program 18 (x 6)		
Enhance		200 µL, 5 min. slow shaking		
Count		KIT 18 (check concentrations from QC certificate)		