A004-201

DELFIA®
hAFP

Time-resolved fluoroimmunoassay

Instructions for use. Reagents for 96 assays

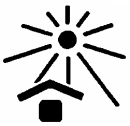
Manufactured by:
PerkinElmer Life and Analytical Sciences, Wallac Oy,
Mustionkatu 6, Turku, Finland

FOR *IN VITRO* DIAGNOSTIC USE

CE

PerkinElmer®
precisely.

SYMBOLS

IVD	For *in vitro* diagnostic use / Pour usage diagnostique *in vitro* / Zur *in vitro* – Diagnostik / Para diagnóstico *in vitro* / Per uso diagnostico *in vitro* / Para uso em diagnóstico *in vitro* / För användning vid *in vitro* -diagnostik / Til *in vitro* diagnostisk brug
LOT	Lot no. / Lot n° / Ch.-Nr. / Nº de lote / No. lotto / No. lote / Lot nr. / Lot. nr.
PN	Packing no. / N° d'emballage / Pack-Nr. / Nº de embalaje / No. confezione / No. de empacotamento / Pack nr. / Pakke nr.
PROD	Product no. / Produit n° / Produkt-Nr. / Nº de referencia / Prodotto n. / No. do produto / Produkt nr. / Produkt nr.
	Expiry date / Date de péremption / Verfallsdatum / Fecha de caducidad / Data di scadenza / Data de validade / Utgångsdatum / Udløbsdato
	Store at / Stocker entre / Lagerung bei / Almacenar a / Conservare a / Estocar a / Förvaras i / Opbevares ved
	Protect from heat and light / A protéger de la chaleur et de la lumière / Vor Wärme- und Lichteinwirkung schützen / Proteger del calor y de la luz / Tenere al riparo dal calore e dalla luce / Mantenha ao abrigo do calor e da luz / Skyddas mot värme och ljus / Beskyttes mod varme og lys
	Contains reagents for 96 tests / Contient des réactifs pour 96 dosages / Enthält Reagenzien für 96 Bestimmungen / Contiene reactivos para 96 ensayos / Contiene reagenti per 96 dosaggi / Contém reagentes para 96 determinações / Innehåller reagens för 96 bestämningar / Indeholder reagenser til 96 bestemmelser
	Note: Read the instructions for use / Remarque: Lire le mode d'emploi / Hinweis: Gebrauchsinformationen beachten / Nota: Leer las instrucciones de uso / Nota: Leggere le istruzioni d'uso / Nota: Leia as instruções para uso / OBS: Läs instruktioner för handhavande / Bemærk: Læs forskriften
	Manufacturer / Fabricant / Hersteller / Fabricante / Produttore / Produzido por / Tillverkare / Producent

DELFIA® hAFP kit

INTENDED USE

This kit is intended for the quantitative determination of human alpha-fetoprotein (hAFP) in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

Human alpha-fetoprotein (hAFP) is a glycoprotein of fetal origin. It is first synthesized by the embryonic yolk sac cells and later by the fetal liver (1), and it diffuses to the maternal blood via the amniotic membranes and reaches the amniotic fluid via fetal urine. hAFP consists of a single polypeptide chain of 590 amino acids and has a molecular weight of approximately 65,000. It has a carbohydrate content of 3 - 4%. The relative proportions of sialic acid, mannose and galactose residues may vary, causing electrophoretic microheterogeneity as well as heterogeneity with respect to lectin binding. The amino acid composition of hAFP displays about 40% homology with human albumin, and they have similar physical and chemical properties, but their immunochemical properties are clearly distinct from each other (2,6,7).

The biological function of hAFP is unknown although several suggestions as to its role have been put forward (2,4,5).

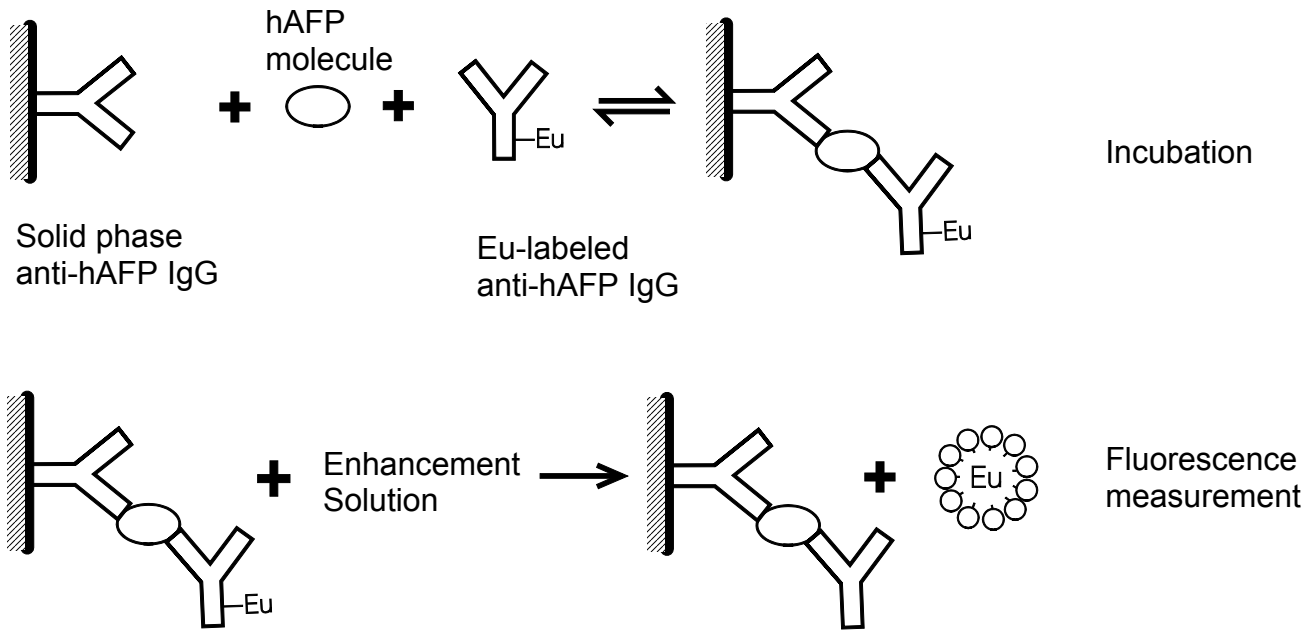
In adult life hAFP may be produced by malignant tumors, especially liver cancer and germ cell tumors. A slight elevation of serum hAFP in patients with hepatitis and cirrhosis has also been observed (3).

Assays of hAFP in serum are important for antenatal detection of fetal abnormalities. hAFP is also determined in the diagnosis and monitoring of hAFP-producing tumors of liver and yolk sac origin.

PRINCIPLES OF THE ASSAY

The DELFIA® hAFP assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the hAFP molecule. The assay can be performed according to protocols involving either one or two incubations. In the one-incubation assay, standards, controls, and serum samples, containing hAFP are reacted simultaneously with immobilized monoclonal antibodies directed against a specific antigenic site on the hAFP molecule and europium-labeled monoclonal antibodies directed against a different specific antigenic site on the same molecule. A two-incubation procedure is recommended to be used for samples which may contain very high levels of hAFP. These samples are first reacted with the immobilized antibody (first incubation), the strips are washed and then the europium-labeled antibodies are reacted with the bound hAFP (second incubation).

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 hAFP in the sample (8,9,10,11).



KIT CONTENTS

Each DELFIA hAFP 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
hAFP Standards (approx. values)	6 vials, 1.1 mL	+2 - +8°C until expiry date stated on the vial label.
A 0 U/mL B 1 U/mL C 10 U/mL D 100 U/mL E 500 U/mL F 1000 U/mL	The exact hAFP concentrations are given on the lot specific quality control certificate included in the kit.	

The ready-for-use standards are in Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, and < 0.1% sodium azide as preservative. The standards have been calibrated against the WHO 1st International Standard (72/225).

Conversion factor: 1 U = 1.21 ng.

Anti-hAFP-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.

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.

A 25-fold concentration of Tris-HCl buffered (pH 7.8) salt solution with Tween 20. Contains Germall II[1] as preservative.

Multibuffer	1 bottle, 50 mL	+2 - +8°C until expiry date stated on the bottle label.

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.

Ready-for-use Enhancement Solution with Triton X-100[2], acetic acid and chelators.

Anti-hAFP Microtitration Strips. 8 x 12 wells coated with antibodies against hAFP (mouse monoclonal)	1 plate	+2 - +8°C until expiry date stated on the label.
Resealable plastic bag for storage of microtitration strips	1 pc	

[1] Germall is a registered trademark of Sutton Laboratories Inc.
[2] Triton is a registered trademark of Rohm and Haas Co.

Lot specific quality control certificate	1 pc

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The DELFIA hAFP kit is part of a complete system of immunodiagnostic 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)
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
- pipettes for dispensing the milliliter volumes of Multibuffer required to prepare the tracer dilution
- distilled water

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. In the one-incubation assay heparin plasma can also be used, but plasma containing EDTA or citrate cannot be used due to chelating effects on europium. The two-incubation assay may be used for serum and heparin plasma samples, and should always be used for plasma samples containing citrate and EDTA. Hemolytic (hemoglobin $\leq$ 480 mg/dL), lipemic (Intralipid[3] $\leq$ 480 mg/dL) and icteric (bilirubin $\leq$ 29 mg/dL) samples do not interfere with the assay.

Heterophilic antibodies in the patient sample may occasionally interfere with the assay, even though specific blocking agents are included in the Multibuffer.

Complement activation may in some rare cases give falsely low results.

If hAFP concentrations exceed (or are expected to exceed) 1000 U/mL, they should be diluted with zero standard or DELFIA Diluent II.

[3] Intralipid is a trademark of Pharmacia & Upjohn.

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 *in vitro* diagnostic use.

This kit should only be used by adequately trained personnel.

Handle all patient 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 (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

One-incubation procedure
Serum or heparin plasma samples only

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-hAFP-Eu tracer solution	**Prepare within one hour of use.**

Prepare the needed volume of tracer dilution by mixing 40 µL of 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.

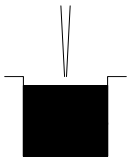
2. Transfer the required number of microtitration strips to a strip frame.

   Note: Open the foil from three sides only and fold it aside leaving the plate-specific information on the package. Store the remaining strips with the desiccant in the resealable plastic bag supplied with the kit.

3. Pipette 25 µL of the hAFP Standards (Std) and patient 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
1st Unk	1st Unk	2nd Unk	2nd Unk	3rd Unk	3rd Unk	etc.						B
												C etc.

4. Add 200 µL of diluted Anti-hAFP-Eu tracer solution 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 for 1 hour (± 10 minutes) at room temperature with **slow** shaking.

6. After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 4 (1 Inc, wash).

7. 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 kit program 4 or MultiCalc® [4] protocol "4 AFP" for automatic measurement and result calculation.

[4] MultiCalc is a registered trademark of PerkinElmer, Inc.

When using VICTOR^2 D start the measurement from the Start Wizard, select "AFP" from Protocols/Kits panel "Tumor" and define the number of plates and samples.

Check the parameter group for program 4 or the MultiCalc protocol "4 AFP". 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	**(Make sure that the hAFP standard**
STANDARD CONC	:	C	**concentrations correspond to those**
STANDARD CONC	:	D	**given on the lot specific quality control**
STANDARD CONC	:	E	**certificate. If this is not the case, enter**
STANDARD CONC	:	F	**the new concentrations.)**
UNKNOWN REPLICATES	:	2	

Two-incubation procedure
Serum or plasma samples

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-hAFP-Eu tracer solution	**Prepare within one hour of use, during the first incubation.**

Prepare the needed volume of tracer dilution by mixing 40 µL of 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.

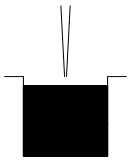
2. Transfer the required number of microtitration strips to a strip frame.

   Note: Open the foil from three sides only and fold it aside leaving the plate-specific information on the package. Store the remaining strips with the desiccant in the resealable plastic bag supplied with the kit.

3. Pipette 25 µL of the hAFP Standards (Std) and patient 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
1st Unk	1st Unk	2nd Unk	2nd Unk	3rd Unk	3rd Unk	etc.						B
												C etc.

4. 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.



5. Incubate for 1 hour (± 10 minutes) at room temperature with **slow** shaking.

6. After the first incubation step, aspirate and wash each strip with the DELFIA Platewash using program 4 (2 Inc, wash 1).

7. Add 200 µL of diluted Anti-hAFP-Eu tracer solution to each well. Pipetting should be as for the Multibuffer in step 4 above.

8. Incubate the frame for 1 hour (± 10 minutes) without shaking at room temperature.

9. After the second incubation step, aspirate and wash each strip with the DELFIA Platewash using program 4 (2 Inc, 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 4 or MultiCalc protocol "4 AFP" for automatic measurement and result calculation.

    When using VICTOR^2 D start the measurement from the Start Wizard, select "AFP" from Protocols/Kits panel "Tumour" and define the number of plates and samples.

    Check the parameter group for program 4 or the MultiCalc protocol "4 AFP". 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	**(Make sure that the hAFP standard**
STANDARD CONC	:	C	**concentrations correspond to those**
STANDARD CONC	:	D	**given on the lot specific quality control**
STANDARD CONC	:	E	**certificate. If this is not the case, enter**
STANDARD CONC	:	F	**the new concentrations.)**
UNKNOWN REPLICATES	:	2	

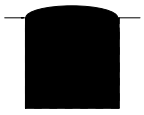
PROCEDURAL NOTES

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 patient 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).

Quality control

The use of control sera is advised to assure the day-to-day validity of results. The controls should be run in the same way as the samples. It is recommended that the laboratory prepares its own serum pools at different levels, or alternatively uses commercial controls, e.g. Lyphochek[5]. A high, a medium and a low level control should be run in each assay; if the assay includes more than one plate, controls should be run on each plate. Patient results should only be reported if control results for the assay meet the laboratory's established criteria for acceptability (13).

We also recommend participation in external quality control schemes.

LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definite clinical judgement should not be based on the results of any single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

Because of the hook effect in the one-incubation procedure, the two-incubation assay is recommended to be used when high values are expected.

Heterophilic antibodies in the patient sample may occasionally interfere with the assay, even though specific blocking agents are included in the Multibuffer.

Complement activation may in some rare cases give falsely low results.

[5] Lyphochek is a registered trademark of Bio-Rad Laboratories Inc.

If hAFP concentrations exceed (or are expected to exceed) 1000 U/mL, they should be diluted with zero standard or DELFIA Diluent II.

Please also refer to the section "PROCEDURAL NOTES".

EXPECTED VALUES[6] AND INTERPRETATION OF RESULTS

Please note that the values mentioned in this section should only be used as a guideline, and each laboratory should establish its own reference range.

The reference range in serum samples when measured from 83 apparently healthy men (aged 24 - 58 years) and from 102 apparently healthy women (aged 23 - 58 years) with the A004-201 DELFIA hAFP kit was 0.8 - 8.7 U/mL. The mean value was 2.99 U/mL.

The lower and upper extremes of the reference range were examined and their confidence intervals were estimated according to IFCC recommended non-parametric statistical treatment (12).

Non-parametric estimates:

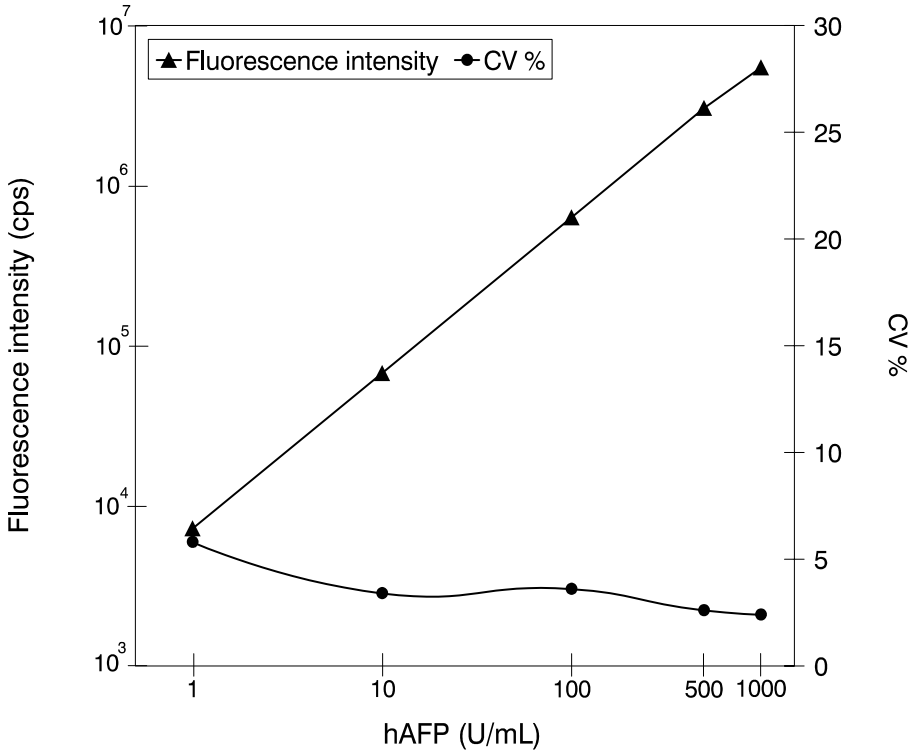
Fraction	Reference limit	0.90-confidence interval
2.5%	0.8 U/mL	0.7 - 1.0 U/mL
97.5%	8.7 U/mL	6.7 - 12.3 U/mL

ANALYTICAL PERFORMANCE CHARACTERISTICS

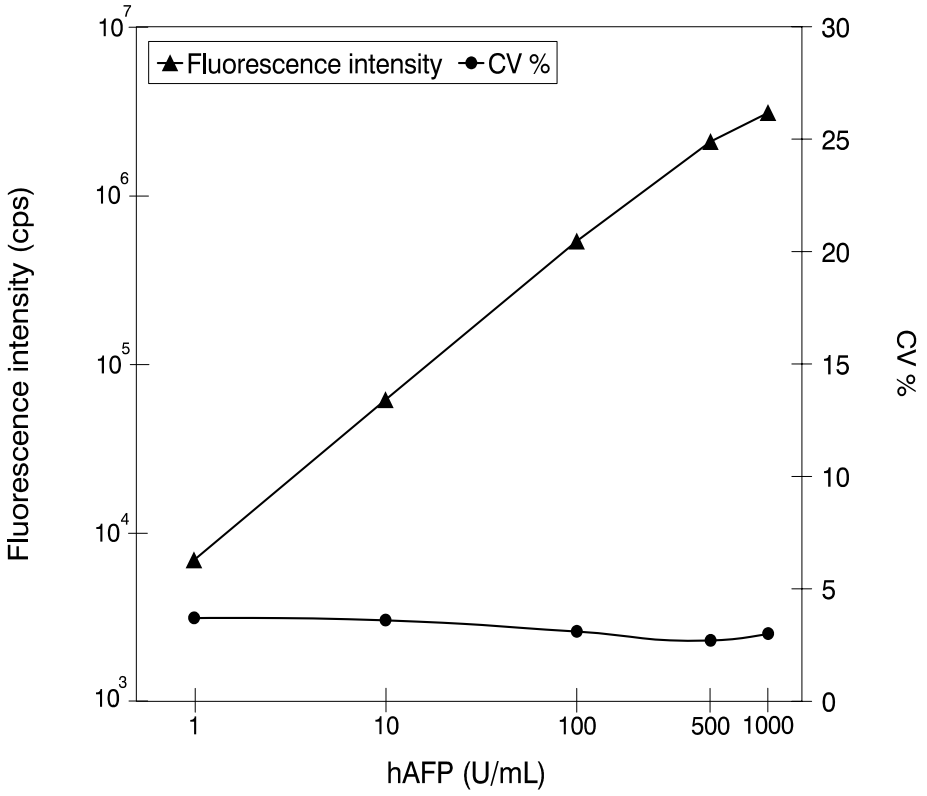
Typical standard curves and precision profiles obtained with the A004-201 DELFIA hAFP assay are shown below. The precision profiles for protocols A and B were calculated from 214 and 216 duplicate measurements of standards and serum specimens, respectively, using the MultiCalc data management program.

[6] Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.

A. One-incubation procedure



B. Two-incubation procedure



Precision[7]: The variation of the DELFIA hAFP assay was determined in 54 runs with 3 replicates, and the analysis of variance approach was used to calculate the following variations:

Results using one-incubation assay procedure:

Serum sample	Total mean value (U/mL)	Intra-assay variation (% CV)	Inter-assay variation (% CV)	Total variation (% CV)
1	2.0	4.0	3.1	5.0
2	20.7	2.6	1.2	2.9
3	348	1.6	2.7	3.2

Results using two-incubation assay procedure:

Serum sample	Total mean value (U/mL)	Intra-assay variation (% CV)	Inter-assay variation (% CV)	Total variation (% CV)
1	1.9	2.0	2.4	3.1
2	20.4	2.5	1.7	3.1
3	349	2.7	2.8	3.9

Analytical sensitivity[8]: The analytical sensitivity of the DELFIA hAFP assay is typically better than 0.1 U/mL, 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).

Recovery[9]: Spiked serum samples were prepared by adding varying levels of concentrated hAFP solution to pooled serum specimens containing a known amount of hAFP. Recoveries for the one-incubation procedure were in the range of 99 - 110% with a mean value of 102 ± 3.0% (SD) (n = 18), and those for the two-incubation procedure were in the range of 94 - 110% with a mean value of 101 ± 5.0% (SD) (n = 18).

Correlation[10]:

A004-201 DELFIA hAFP one-incubation procedure vs. two-incubation procedure:

The A004-201 DELFIA hAFP one-incubation procedure (y) was compared with the A004-201 DELFIA hAFP two-incubation procedure (x) using patient specimens in the range of 2 - 895 U/mL hAFP. The correlation was found to be:

$$y = 0.95x + 5.34; \quad r = 1.00 \quad (n = 80)$$

[7] Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.
[8] as above
[9] as above
[10] as above

One-incubation procedures A004-201 DELFIA hAFP vs. 1244-004 DELFIA hAFP:

The A004-201 DELFIA hAFP one-incubation procedure (y) was compared with the 1244-004 DELFIA hAFP one-incubation procedure (x) using patient specimens in the range of 2 - 733 U/mL hAFP. The correlation was found to be:

$y = 1.02x - 2.67$; $r = 1.00$ $(n = 80)$

Two-incubation procedures A004-201 DELFIA hAFP vs. 1244-004 DELFIA hAFP:

The A004-201 DELFIA hAFP two-incubation procedure (y) was compared with the 1244-004 DELFIA hAFP two-incubation procedure (x) using patient specimens in the range of 1 - 740 U/mL hAFP. The correlation was found to be:

$y = 1.09x - 4.87$; $r = 1.00$ $(n = 80)$

One-incubation procedures A004-201 DELFIA hAFP vs. B004-201 AutoDELFIA hAFP:

The A004-201 DELFIA hAFP one-incubation procedure (y) was compared with the B004-201 AutoDELFIA hAFP one-incubation procedure (x) using patient specimens in the range of 2 - 805 U/mL hAFP. The correlation was found to be:

$y = 0.95x + 5.13$; $r = 1.00$ $(n = 80)$

Two-incubation procedures A004-201 DELFIA hAFP vs. B004-201 AutoDELFIA hAFP:

The A004-201 DELFIA hAFP two-incubation procedure (y) was compared with the B004-201 AutoDELFIA hAFP two-incubation procedure (x) using patient specimens in the range of 2 - 700 U/mL hAFP. The correlation was found to be:

$y = 1.05x + 3.73$; $r = 1.00$ $(n = 79)$

Cross reactivity[11]: Physiologically found serum albumin concentrations do not interfere with the DELFIA hAFP determination.

Hook effect[12]: One-incubation procedure: No hook effect has been found for concentrations up to 5 000 U/mL. Two-incubation procedure: No hook effect has been found for concentrations up to 50 000 U/mL.

[11] Study performed at PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.
[12] as above

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.

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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

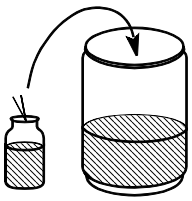
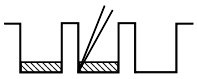
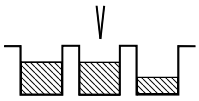
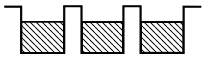
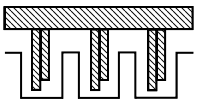
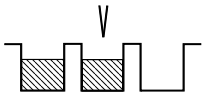
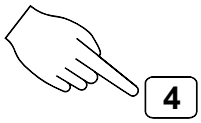
Sweden: 8102753-4

USA: 4,565,790, 4,808,541

Last revision December 2005

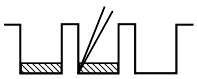
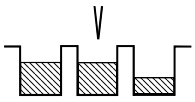
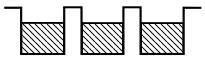
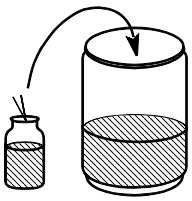
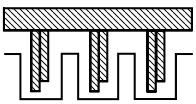
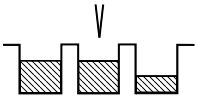
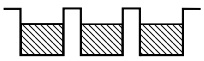
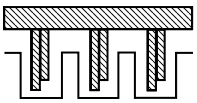
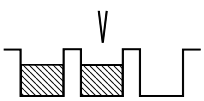
DELFIA® hAFP kit

Summary Protocol Sheet
One-incubation Procedure

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

DELFIA® hAFP kit

Summary Protocol Sheet
Two-incubation Procedure

<table>
<tr><td>Add standards and unknowns</td><td>  </td><td colspan="3">25 µL</td></tr>
<tr><td>Add buffer</td><td>  </td><td colspan="3">200 µL</td></tr>
<tr><td>Incubate</td><td>  </td><td colspan="3">1 h slow shaking at RT</td></tr>
<tr><td>Dilute tracer (see table)</td><td>  </td><td colspan="3"><table><tr><th>Strips</th><th>Tracer stock solution (µL)</th><th>Buffer (mL)</th></tr><tr><td>1</td><td>40</td><td>3</td></tr><tr><td>2</td><td>80</td><td>6</td></tr><tr><td>3</td><td>120</td><td>9</td></tr><tr><td>4</td><td>160</td><td>12</td></tr><tr><td>5</td><td>200</td><td>15</td></tr><tr><td>6</td><td>240</td><td>18</td></tr><tr><td>7</td><td>280</td><td>21</td></tr><tr><td>8</td><td>320</td><td>24</td></tr></table></td></tr>
<tr><td>Wash</td><td>  </td><td colspan="3">Program 4 (x 2)</td></tr>
<tr><td>Add tracer dilution</td><td>  </td><td colspan="3">200 µL</td></tr>
<tr><td>Incubate</td><td>  </td><td colspan="3">1 h without shaking at RT</td></tr>
<tr><td>Wash</td><td>  </td><td colspan="3">Program 4 (x 6)</td></tr>
<tr><td>Enhance</td><td>  </td><td colspan="3">200 µL,
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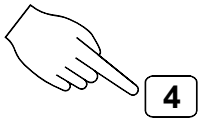
<tr><td>Count</td><td>  </td><td colspan="3">KIT 4
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