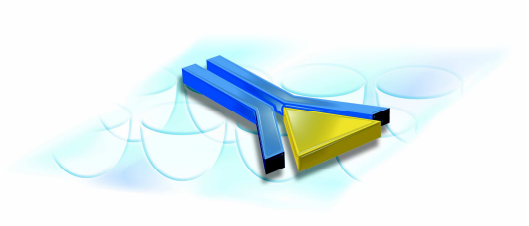
NovaTec
Immundiagnostica GmbH



AFP

Enzyme immunoassay for the quantitative
determination of AFP in human serum or plasma

Only for in-vitro diagnostic use

CE

Product Number: DNOV033 (96 Determinations)

Digitally signed by Bolea Petru
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1. INTRODUCTION

Alpha Fetoprotein (AFP) is a 68 kDa glycoprotein, which is normally only produced in the fetus during its development. It is a normally produced by the liver and yolk sac of the fetus.
AFP levels decrease soon after birth and probably has no function in normal adults. It binds the hormone estradiol to keep it from affecting the fetal brain. Its measurement during pregnancy has been useful to detect certain abnormalities - specifically, if high levels of AFP are found in amniotic fluid, it can indicate a developmental defect in the baby.
In some patients who are not pregnant a tumor can produce AFP, thus it can be used as a tumour marker. AFP is the main tumour marker (along with HCG) to diagnose testicular cancer and its values over time can have significant effect on the treatment plan.
Like all tumour markers, the detection of AFP by itself is not diagnostic of anything, although if it is detected it is certainly advisable to rule out the diseases could cause levels to rise. The primary reason tumor markers are used are to measure the success of a treatment (e.g. chemotherapy), if levels of AFP are going down, it is an indication that a disease is improving. New research exhibits that an isoform of AFP which binds Lens culinaris agglutinin (AFP-L3) can be particularly useful in early identification of aggressive tumors associated with hepatocellular carcinoma (HCC).

2. INTENDED USE

Immunoenzymatic colorimetric method (ELISA) for quantitative determination of AFP in serum or plasma.

3. PRINCIPLE OF THE ASSAY

The AFP assay is based on simultaneous binding of human AFP to two monoclonal antibodies; one is immobilized on the microplate, the other is soluble and conjugated with horseradish peroxidase (HRP). Microtiter strip wells are precoated with anti-AFP IgG antibodies. AFP in samples and standards binds to the immobilised antibodies on the surface of the microtiter wells and the second, soluble anti-AFP antibody-enzyme conjugate binds to the immobile antibody-AFP-complex during the first incubation. Afterwards a bound/free separation is performed by solid-phase washing. The immune complex is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of this product is proportional to the amount of AFP in samples and standards. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Anti-AFP IgG Coated Wells:** 12 breakapart 8-well snap-off strips coated with anti-AFP IgG; in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Anti-AFP-HRP conjugate conc.:** 1 bottle containing 0.3 ml of horseradish peroxidase labelled anti-AFP antibodies.
- **TMB Substrate Solution**: 1 bottle containing 12 ml 3, 3´, 5, 5´-tetramethylbenzidine (H_2O_2-TMB 0.25g/l) (avoid any skin contact).
- **Incubation buffer**: 1 bottle containing 50 ml phosphate buffer 50 mM, pH 7.4, BSA 1 g/l.
- **Wash solution 50x conc**.: 1 bottle containing 20 ml (NaCl 9 g/l, Tween20 1 g/l)
- **AFP Standards:** 5 bottles, 1 ml each. The standards are calibrated against the (WHO 2nd IRP 72/225) and have approximately the following concentrations:

  Standard 0: 0 ng/ml
  Standard 1: 5 ng/ml
  Standard 2: 20 ng/ml
  Standard 3: 80 ng/ml
  Standard 4: 200 ng/ml

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with anti-AFP IgG antibodies. Store at 2…8 °C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.*

6.2. Anti-AFP-HRP Conjugate

The bottle contains 0.3 ml of a concentrated solution with anti-AFP antibodies conjugated with horseradish peroxidase.
Dilute immediately before use:
Add 10 µl concentrated conjugate to 1.0 ml of Incubation Buffer.
Mix gently for 5 min with a vortex mixer.
Stable for 3 hours at room temperature.

6.3. Standards

Each of the 5 vials contains 1 ml standard solution of the concentration mentioned in 4.1. The standards are ready to use. *After first use the standard solutions are still stabile for another 6 months if stored at 2…8 °C.*

6.4. TMB Substrate Solution

The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stabile for another 6 months if stored at 2…8 °C.*

6.5. Stop Solution

The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.
After first use stable until expiry date.

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (heparin, EDTA) samples with this assay. If the assay is performed within 48 hours after sample collection, the specimens should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*
Samples with concentration over 200 ng/ml should be diluted 1/1 with Incubation buffer.

7.1. Precaution

- The reagents contain Proclin 300^{R} (0.01%) as a preservative.
- Do not use heavily haemolysed samples.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- This method allows the determination of AFP from 5 ng/ml to 200 ng/ml.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well	(e.g. A1)	for the substrate blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(eg. F1+G1)	for standard 2
2 wells	(eg. H1+A2)	for standard 3
2 wells	(eg. B2+C2)	for standard 4

It is recommended to determine standards and patient samples in duplicate.
Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 25 µl standards and samples into their respective wells. Add 50 µl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour at room temperature (+22…+28°C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100 µl TMB Substrate Solution into all wells.
6. **Incubate for exactly 15 min at room temperature (+22…+28°C) in the dark.**
7. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue colour developed during the incubation turns into yellow.*
8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

9. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of AFP for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

10. RESULTS

10.1. Calculation

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample. Subtract the mean absorbance value of the zero standard from the mean absorbance values of standards and samples. Plot the mean value of absorbance of the standards (Em) against concentration. Draw the best-fit curve through the plotted points. (Es: Four Parameter Logistic). Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

10.2. Reference values

In a study conducted with apparently normal healthy adults, using NovaTec AFP, the following results were observed:

Population	0-10 ng/ml	20 ng/ml	30 ng/ml
Males	82	2	1
Females	55	1	1

In a study conducted with nonseminomatous testicular cancer patient using AFP, the following values were observed:

Population	0-10 ng/ml	10-100 ng/ml	>100 ng/ml
Males	4	5	3

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Sensitivity

The lowest detectable concentration of AFP that can be distinguished from the standard 0 is 0,1 ng/ml at the 95 % confidence limit.

11.2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham:

AFP	100.0 %
b-HCG	0.01 %
HCG	0.01 %
LH	0.01 %
FSH	0.01 %
TSH	0.01 %
Ibuprofen	--
Caffeine	--
Acetylsalicilic acid	--
Theomobrine	--
Megestrol acetate	--

11.3. Precision

Intra Assay Variation
Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 6.6%.

Inter Assay Variation
Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 6.3%.

11.4. Accuracy

The recovery of 12.5 – 25 – 50 – 100 ng/ml of AFP added to sample gave an average value (±SD) of 99.5% ± 3.7% with reference to the original concentrations.

11.5. Correlation

The NovaTec AFP ELISA was compared to another commercially available AFP assay. Serum samples of 32 females and 4 males were analysed according in both test systems.
The linear regression curve was calculated

$y = 1.04\ x - 0.41$
$r = 0.99\ (r^2 = 0.98)$

11.6. Hook Effect

The AFP ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 4 000 ng/ml.

12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Do not use heavily haemolysed or highly lipemic samples.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
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WARNING:	In the used concentration Proclin 300^R has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE

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14. ORDERING INFORMATION

Prod. No.:	DNOV033	AFP Determination (96 Determinations)

SCHEME OF THE ASSAY

AFP

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate blank	Standard 0	Standard 1	Standard 2	Standard 3	Standard 4	Sample
Standard 0	-	25 µl	-	-	-	-	-
Standard 1	-	-	25 µl	-	-	-	-
Standard 2	-	-	-	25 µl	-	-	-
Standard 3	-	-	-	-	25 µl	-	-
Standard 4	-	-	-	-	-	25 µl	-
Sample	-	-	-	-	-	-	25 µl
Conjugate	-	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
Cover wells with foil supplied in the kit **Incubate for 1 hour at room temperature** Wash each well three times with 300 µl diluted wash solution							
TMB Substrate	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature in the dark							
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm							

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