CE

DiaMetra
care for quality

DCM012-13
Ed. 07/2024

AFP ELISA

for routine analysis

Direct immunoenzymatic determination of AFP in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO012

INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of AFP concentration in human serum or plasma.
AFP ELISA kit is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE

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. PRINCIPLE

AFP ELISA test is based on simultaneous binding of human AFP to two monoclonal antibodies, one immobilized on microwell plates, the other conjugated with horseradish peroxidase (HRP).
After incubation, the separation bound-free is obtained with a simple solid-phase washing.
Then the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blu color that changes into yellow when the Stop Solution (H_2SO_4) is added.
The colour intensity is proportional to the AFP concentration in the sample.
The AFP concentration in the sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1 mL each)
ProClin >0.0015% and BSA 0.1%

CAL0	REF DCE002/1206-0
CAL1	REF DCE002/1207-0
CAL2	REF DCE002/1208-0
CAL3	REF DCE002/1209-0
CAL4	REF DCE002/1210-0

2. Control (1 vial, 1 mL)
ProClin >0.0015% and BSA 0.1%
Concentration of Control is indicated on the Certificate of Analysis — REF DCE045/1203-0

3. Incubation Buffer (1 vial, 50 mL)
Phosphate buffer 50 mM pH 7.4; BSA 1 g/L. ProClin >0.0015% — REF DCE001/1401-0

4. Conjugate (1 vial, 1 mL)
Monoclonal anti AFP antibody conjugated with Horseradish peroxidase (HRP). ProClin >0.0015% and BSA 0.1% — REF DCE002/1202-0

5. Coated Microplate (1 breakable microplate)
Monoclonal anti AFP antibody adsorbed on microplate — REF DCE002/1203-0

6. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact).* ProClin <0.0015% — REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)* — REF DCE005-0

8. 50X Conc. Wash Solution (1 vial, 20 mL)
NaCl 45 g/L; Tween-20 55 g/L. ProClin >0.0015% — REF DCE006-0

3.2. Reagents necessary not supplied

Distilled water.

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3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm, 620-630 nm)

Note

Store all reagents at 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents (calibrators, control, incubation buffer, conjugate and wash solution) contain small amounts of ProClin™ 300 (>0.0015%, <0.06%) as preservative. Avoid contact with skin or mucosa.
- Classification according to Regulation (EC) No. 1272/2008 [CLP]
  Skin sensitivity, Category 1



Contains: ProClin 300

Warning

Hazard statements:
H317 - May cause an allergic skin reaction.

Precautionary statements:
P261 - Avoid breathing dust / fume / gas / mist / vapours / spray.
P280 - Wear protective gloves/ protective clothing / eye protection / face protection / hearing protection.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.

- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of AFP from 5 ng/mL to 200 ng/mL.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

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6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_4)

The Calibrators are ready to use, are calibrated against the WHO 1st IS 72/225 and have the following concentrations:

	C_0	C_1	C_2	C_3	C_4
ng/mL	0	5	20	80	200

The Calibrators are stable until the expiry date printed on the label. Once opened, the calibrators are stable six months at 2-8°C

6.2. Preparation of Diluted Conjugate

Prepare immediately before use.
Add 10 µL of Conjugate (reagent 4) to 1 mL of Incubation Buffer (reagent 3). The quantity of diluted conjugate is proportional at the number of the tests.
Mix gently for 5 minutes, with a rotating mixer.
Stable for 3 hours at room temperature (22÷28°C).

6.3. Preparation of the Sample

AFP determination should be done in human serum or plasma.
Specimen can be stored at 2÷8°C for at short time (max two days). For longer storage the specimen should be frozen at -20°C. Avoid repeated freezing and thawing.
For sample with concentration over 200 ng/mL dilute the sample with Incubation buffer.
The Control is ready to use.

6.4. Preparation of Wash Solution

Dilute the content of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2÷8°C.

6.5. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Calibrator C_0-C_4	**25 µL**		
Sample/ Control		**25 µL**	
Diluted Conjugate	**50 µL**	**50 µL**	
Incubate at room temperature (22÷28°C) for 1 hour. Remove the contents from each well; wash the wells 3 times with 300 µL of diluited wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22÷28°C) for 15 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. 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 calibration 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.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C_0-C_4) and of each sample.

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8.2. Calibration curve

Plot the values of absorbance (Em) of the calibrators (C_0-C_4) against concentration. Draw the best-fit curve through the plotted points. (Es: Four Parameter Logistic).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

9. REFERENCE VALUES

In a study conducted with apparently normal healthy adults, using Dia.Metra 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

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

Within run variation was determined by replicate (16x) the measurement of three different control sera in one assay. The within assay variability is ≤ 6.4%.

10.1.2. Inter Assay Variation

Between run variation was determined by replicate (16x) the measurements of three different control sera in different lots. The between assay variability is ≤ 6.5%.

10.2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

AFP	100 %
βHCG	0.01 %
HCG	0.01 %
hLH	0.01 %
hFSH	0.01 %
hTSH	0.01 %

10.3. Accuracy

The recovery of 12.5 – 25 – 50 ng/mL of AFP added to sample gave an average value (±SD) of 98.33% ± 4.43% with reference to the original concentrations.

10.4. Sensitivity

The lowest detectable concentration of AFP that can be distinguished from the Calibrator 0 is 0.35 ng/mL at the 95% confidence limit.

10.5. Correlation with RIA

Dia.Metra 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:

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

10.6. Hook Effect

The AFP ELISA shows no Hook Effect up to 400 ng/mL.

For samples from patients suspected to be affected by oncological conditions it is recommended to dilute the sample 1:10 and 1:100 in incubation buffer. If results with these dilutions is not consistent, a further dilution of 1:1000 should be performed to determine AFP level within the patient sample.

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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Dia.Metra S.r.l.
Via Pozzuolo 14,
06038 SPELLO (PG) Italy
Tel. +39-0742-24851
Fax +39-0742-316197
E-mail: info@diametra.com

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DiaMetra	Packaging Information Sheet	Mod. PIS000-2

IVD	DE	*In vitro* Diagnostikum		DE	Hergestellt von
	ES	Producto sanitario para diagnóstico *In vitro*		ES	Elaborado por
	FR	Dispositif medical de diagnostic *in vitro*		FR	Fabriqué par
	EN	*In vitro* Diagnostic Medical Device		EN	Manufacturer
	IT	Dispositivo medico-diagnostico *in vitro*		IT	Produttore
	PT	Dispositivos medicos de diagnostico *in vitro*		PT	Produzido por
	DE	Achtung, Begleitdokumente	 yyyy-mm	DE	Herstellungs datum
	ES	Precaución, consulte los documentos adjuntos		ES	Fecha de fabricacion
	FR	Attention, veuillez consulter les documents d'accompagnement		FR	Date de fabrication
	EN	Caution, consult accompanying documents		EN	Date of manufacture
	IT	Attenzione, consultare la documentazione allegata		IT	Data di produzione
	PT	Atenção,consultar os documentos de acompanhamento		PT	Data de produção
 yyyy-mm-dd	DE	Verwendbar bis		DE	Biogefährdung
	ES	Establa hasta (usar antes de último día del mes)		ES	Riesco biológico
	FR	Utiliser avant (dernier jour du mois indiqué)		FR	Risque biologique
	EN	Use by (last day of the month)		EN	Biological risk
	IT	Utilizzare prima del (ultimo giorno del mese)		IT	Rischio biologico
	PT	Utilizar (antes ultimo dia do mês)		PT	Risco biológico
	DE	Gebrauchsanweisung beachten	LOT	DE	Chargenbezeichnung
	ES	Consultar las instrucciones		ES	Codigo de lote
	FR	Consulter le mode d'emploi		FR	Numero de lot
	EN	Consult instructions for use		EN	Batch code
	IT	Consultare le istruzioni per l'uso		IT	Codice del lotto
	PT	Consultar instruções para uso		PT	Codigo do lote
 Σ = xx	DE	Ausreichend für "n" Tests	CONT	DE	Inhalt
	ES	Contenido suficiente para "n" tests		ES	Contenido del estuche
	FR	Contenu suffisant pour "n" tests		FR	Contenu du coffret
	EN	Contains sufficient for "n" tests		EN	Contents of kit
	IT	Contenuto sufficiente per "n" saggi		IT	Contenuto del kit
	PT	Contém o suficiente para "n" testes		PT	Conteúdo do kit
	DE	Temperaturbereich	REF	DE	Bestellnummer
	ES	Límitaciôn de temperatura		ES	Nûmero de catálogo
	FR	Limites de température de conservation		FR	Réferéncès du catalogue
	EN	Temperature limitation		EN	Catalogue number
	IT	Limiti di temperatura		IT	Numero di Catalogo
	PT	Temperaturas limites de conservação		PT	Número do catálogo
	DE	Vor direkter sonneneinstrahlung schützen			
	ES	Mantener alejado de la luz solar			
	FR	Tenir à l'écart de la lumière du soleil			
	EN	Keep away from sunlight			
	IT	Tenere lontano dalla luce solare			
	PT	Mantenha longe da luz solar			

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