



DCM012-11  
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## 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

Eagle Biosciences AFP ELISA Assay Kit is an immunoenzymatic colorimetric method for quantitative determination of AFP concentration in human serum or plasma. AFP kit is intended for research use only not intended for diagnostic procedures.

### 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 Assay Kit 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<sub>2</sub>O<sub>2</sub>) and the TMB Substrate and

develops a blue color that changes into yellow when the Stop Solution (H<sub>2</sub>SO<sub>4</sub>) is added.

The color 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. AFP Calibrators (5 vials, 1 mL each)

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. AFP Control (1 vial, 1 mL)

Concentration of Control is Lot-specific and is indicated on Quality Control Report

**REF** DCE045/1203-0

3. Incubation Buffer (1 vial, 50 mL)

Phosphate buffer 50 mM pH 7.4; BSA 1 g/L

**REF** DCE001/1201-0

4. Conjugate (1 vial, 1 mL)

Monoclonal Anti AFP conjugated with Horseradish peroxidase (HRP)

**REF** DCE002/1202-0

5. Coated Microplate (1 breakable microplate)

Monoclonal Anti AFP adsorbed on microplate

**REF** DCE002/1203-0

6. TMB Substrate (1 vial, 15 mL)

H<sub>2</sub>O<sub>2</sub>-TMB 0.26 g/L

(avoid any skin contact)

**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

**REF** DCE006-0

#### 3.2. Reagents necessary not supplied

Distilled water.

#### 3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 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.

**4. WARNINGS**

- This AFP ELISA Assay Kit is intended for research 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 of the AFP ELISA Assay Kit contain small amounts of Proclin 300<sup>R</sup> as preservative. Avoid the contact with skin or mucosa.
- 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<sub>2</sub>O<sub>2</sub> to directed sunlight, metals or oxidants. Do not freeze the solution.
- This AFP ELISA Assay Kit 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 of the AFP ELISA Assay Kit 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 AFP ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange AFP ELISA Assay 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.
- 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 lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

**6. PROCEDURE****6.1. Preparation of the Calibrators (C<sub>0</sub>...C<sub>4</sub>)**

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

	C <sub>0</sub>	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>
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).**
- 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<sub>0</sub>-C<sub>4</sub>), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Calibrator C <sub>0</sub> -C <sub>4</sub>	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 diluted wash solution.			
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 Blank.			

#### 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 (E<sub>m</sub>) for each point of the calibration curve (C<sub>0</sub>-C<sub>4</sub>) and of each sample.

### 8.2. Calibration curve

Plot the values of absorbance (E<sub>m</sub>) of the calibrators (C<sub>0</sub>-C<sub>4</sub>) 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 Diametra AFP, the following results were observed:

Population	0-10 ng/mL	20 ng/mL	30 ng/mL
<b>Males</b>	82	2	1
<b>Females</b>	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
<b>Males</b>	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 Manufacturer 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 ( $\pm$ SD) of 98.33%  $\pm$  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

Diametra 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, a competitive enzyme immunoassay, shows no Hook Effect up to 4000 ng/mL.

## 11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

## BIBLIOGRAPHY

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## **ERROR POSSIBLE CAUSES / SUGGESTIONS**

### **No colorimetric reaction**

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

### **Too low reaction (too low ODs)**

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

### **Too high reaction (too high ODs)**

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

### **Unexplainable outliers**

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation