Atlas Medical

ALPHA-FETOPROTEIN (AFP) ENZYME IMMUNOASSAY TEST

IVD *For In Vitro professional Use Only*

2°C 8°C *Store at 2 to 8°C.*

CE

Σ ***96 Tests***

INTENDED USE

The purpose of the AFP ELISA Test is intended for quantitative determination of Alpha Fetoprotein (AFP) in human serum and in amniotic fluid.

INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70,000 daltons. AFP is normally produced during fetal and neonatal development by the liver, yolksac, and in small concentrations decrease rapidly, and by the second year of life thereafter only trace amounts are normally detected in serum.

Elevation of serum AFP to abnormally high values occurs in several malignant diseases, most notably nonseminomatous testicular cancer and primary hepatocullular carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease. Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonseminomatous elements, but not in patients with pure seminoma.

In addition, elevated serum AFP concentrations have been measured in patients with other noncancerous diseases, including ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis, and pregnant women. Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

The AFP assay provides an enzyme immunoassay system with high sensitivity for human AFP measurement in serum and amniotic fluid.

PRINCIPLE OF THE TEST

Sandwich principle. Total duration of assay: 50 minutes.

The AFP assay is a quantitative solid phase enzyme-linked immunosorbent assay (ELISA).The wells are coated with anti-AFP antibodies. The samples, Standards and Controls are incubated in the wells with enzyme conjugate which is another antibody directed toward a different region of AFP molecules and chemically conjugated with horseradish peroxidase. Unbound enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the AFP present in the Samples, Standards and Controls, the intensity of color developed is proportional to the concentration of AFP in the serum, The optical density of the colored samples is read with a microplate reader at 450 nm.

MATERIALS AND COMPONENTS

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

1. **MICROPLATE:** One 96-well black microplate.
2. **CALIBRATORS:** Six white cap vials **(1 ml each)** of references for AFP Antigen at levels of 0 (A), 10.0 (B), 20.0 (C), 50.0 (D), 100.0 (E) and 400.0 (F) ng/ml. Store at 2-8°C.
3. **Enzyme CONJUGATE: 1x11ml red cap vial**
4. **WASH BUFFER (40X): 1x25ml white bottle**
5. **SUBSTRATE SOLUTION: 1x11ml brown cap vial**
6. **STOP SOLUTION: 1x6ml yellow cap vial**
7. **ONE PACKAGE INSERT**
8. **PLATE Lid**: 2 pieces

Materials required but not provided:

- Distilled water.
- Microplate reader with 450nm wavelength absorbent capability.
- Absorbent paper.
- Micropipettes and multichannel micropipettes delivering 50 µl with a precision of better than 1.5%.
- Plate shaker.
- Incubator.
- Microplate washer.

PRECAUTIONS

1. For In Vitro diagnostic use only.
2. 1 IU/ml equivalent 1 ng/ml.p
3. Read the instruction carefully prior to use and do the test according to the instruction strictly.
4. Do not exchange reagents from different lots or use reagents from other commercially available kits.
5. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
6. Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use.
7. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
8. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
9. The time of reaction and coloration should be strictly controlleds.
10. Ensure that the bottle cops of the reagents are correct.

STORAGE AND STABILITY

- Store at 2-8°C.
- Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
- Seal and return unused calibrators to 2-8 °C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles.
- Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

SPECIMENS COLLECTION

1. Collect serum (not fasting blood) according to the conventional method.
2. Avoid grossly hemolytic samples.
3. Ensure that the specimen reach room temperature (18-26°C) prior to being tested.
4. Specimen should be fresh serum. If testing will be delayed more than 24 hours, serum should be stored below -20°C.
5. Turbidness or precipitation in the specimen should be removed by centrifugation before testing.
6. Freeze only once.

QUALITY CONTROL

- Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. There controls should be treated as unknowns and values determined in every test procedure performed.
- The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

TEST PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C).

1. Secure the desired number of coated wells in the holder.
2. Place **100ul** of **Calibrators**, or Serum samples to each well.
3. Incubate **at 37℃ for 20 minutes.**
4. Place **350 µl** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

5. Dispense **100ul** of **Enzyme** into each well and incubate **for 20 minutes at 37℃.**
6. Place **350 µl** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of **5 washes**. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
7. Place **100 µl** of **substrate** to each well.
8. **Incubate** at **ambient temperature (18-25℃)** in the dark for reaction for **10 minutes.** Do not shake the plate after substate addition.
9. Place **50 µl** of **stop solution** to each well.
10. Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
11. Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

CALCULATION OF RESULTS

Any microwell reader capable of determining at 450 nm may be used. The AFP value of patient is obtained as follows:

- Plot the concentration (X) of each Reference Standards against its absorbance (Y) on linear graph paper.
- Obtain the value of patient AFP by reference to the Standard Curve. For example: (This data is for demonstration purposes only and must not be used in place of data generated for each assay).

LIMITATIONS

1. For diagnostic purposes, the AFP values should be used as an adjunct to other data available to the physician.
2. The AFP quantitative assay kit has been designed to avoid the “hook effect”.
3. Samples with AFP levels above 250 ng/mL should be diluted to obtain an accurate value.

EXPECTED VALUES

- Each laboratory should determine its own normal and abnormal ranges.
- In a small scale (n=220 AFP concentration in the normal sera were obtained to be less than 12 ng/ml.
- The following reported value can be used as a guide from literature : the concentration of AFP in hepatocelluar carcinoma and germ cell tumor varies from the range of normal up to several million ng/ml. After surgical resection, the serum AFP may drop to normal range or somewhat above it.
- According to literature, AFP level is elevated up to 600 ng/ml in material serum during normal pregnancy. Material serum AFP above 2.5 times the normal median for weeks 16 to 18 of pregnancy detected 88% of cases of anencephaly and 79% of cases of open spinal bifide.

REFERENCE

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry