ALPHA-FETOPROTEIN (AFP) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: BC-1009

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Enzyme Immunoassay for the Quantitative Determination of Alpha-Fetoprotein (AFP) in Human Serum

FOR INVESTIGATIONAL USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

AFP Enzyme Immunoassay

INTENDED USE

For the quantitative determination of the Cancer Antigen AFP concentration in human serum.

INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weigh of approximately 70,000 daltons. AFP is normally produced during fetal and neonatal development by the liver, yolksac, and in small concentrations by the gastrointestinal tract. After birth, serum AFP concentrations decrease rapidly, and by the second year of life and 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 hepatocellular 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 cirrhosis. Elevated serum AFP concentrations are also observed in pregnant women. Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

PRINCIPLE OF THE TEST

The AFP ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a goat anti-AFP antibody directed against intact AFP for solid phase immobilization (on the microtiter wells). A monoclonal anti-AFP antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react first with the immobilized rabbit antibody for 30 minutes. The wells are washed to remove any unbound antigen. The monoclonal-HRP conjugate is then reacted with the immobilized antigen for 30 minutes at room temperature resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for

20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of AFP is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kits:

- Goat anti-AFP coated microtiter plate with 96 wells.
- Zero Buffer, 13 ml.
- Reference standard set, contains 0, 5, 20, 50, 150, and 300 ng/ml (WHO, 72/225) AFP, lyophilized.
- Enzyme Conjugate Reagent, 18 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes: 20 µl, 100 µl, and 150 µl, and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Zero Buffer into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.

5. Incubate at room temperature (18-25°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100 µl TMB Reagent into each well. Gentle mix for 10 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
18. Read optical density at 450 nm with a microtiter reader ***within 15 minutes.***

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of AFP in ng/ml from the standard curve.

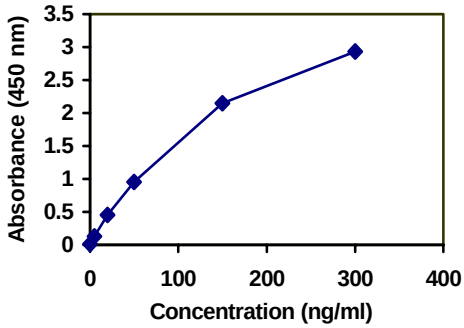
EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against AFP concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

AFP (ng/ml)	Absorbance (450 nm)
0	0.012
5	0.127
20	0.455
50	0.952
150	2.150
300	2.932

EXPECTED VALUES AND SENSITIVITY

In high-risk patients, AFP values between 100 and 350 ng/ml suggest a diagnosis of hepatocellular carcinoma, and levels over 350 ng/ml usually indicate the disease. Approximately 97% of the healthy subjects have AFP levels less than 8.5 ng/ml. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of AFP by this assay is



estimated to be 2.0 ng/ml.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

REFERENCES

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