

VISUAL ALPHA-FETOPROTEIN (AFP) ENZYME IMMUNOASSAY TEST KIT

Catalog Number: BC-1063



BioCheck, Inc
323 Vintage Park Dr.
Foster City, CA 94404

Visual ELISA Test for the Qualitative Determination of Alpha-Fetoprotein (AFP) in Human Serum

FOR INVESTIGATIONAL USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

BioCheck Visual AFP Enzyme Immunoassay

INTENDED USE

For the qualitative determination of the Cancer Antigen AFP concentration in human serum

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, yolk sac, 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 rabbit anti-AFP antibody directed against intact AFP for solid phase immobilization (on the microtiter wells). A monoclonal anti-AFP antibody conjugated to horseradish peroxidase (HRPO) is in the antibody-enzyme conjugate solution. The test sample is allowed to

react first with the immobilized rabbit antibody at 37°C for 15 minutes. The wells are washed to remove any unbound antigen. The monoclonal-HRPO conjugate is then reacted with the immobilized antigen at 37° for 15 minutes 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 5 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. Comparing the color intensity of patient samples with that of the provided known reference, the amount of AFP can be visually estimated to be greater or less than 20 ng/ml.

REAGENTS

Materials provided with the kits:

- Rabbit anti-AFP coated microtiter plate with 96 wells.
- Enzyme Conjugate Reagent, 7 ml.
- AFP Reference Standard: containing 20 ng/ml (WHO, 72/225) AFP, lyophilized.
- AFP Reference Standard: containing 300 ng/ml (WHO, 72/225) AFP, lyophilized.
- TMB Reagent (One-Step), 7 ml.
- Stop Solution (1N HCl), 7 ml.

Materials required but not provided:

- Precision pipettes: 0.05 and 1.0 ml
- Disposable pipette tips.
- Distilled water.
- Absorbent paper or paper towel.

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.

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 60 days when stored sealed at 2-8°C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 μ l of Standards and patient samples into appropriate wells.
3. Incubate at 37°C for 15 minutes.
4. Remove the incubation mixture by flicking plate content into a sink, followed by rinsing the wells 5 times with **distilled water**.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Dispense 1 drop (50 μ l) of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
7. Incubate at 37°C for 15 minutes.
8. Repeat procedures 4 and 5.
9. Dispense 1 drop (50 μ l) of TMB Reagent into each well. Gentle mix for 5 seconds.
10. Incubate at room temperature for 5 minutes.
11. Add 1 drop (50 μ l) of Stop Solution (1N HCl) into each well.
12. Gently mix for 10 seconds to ensure a complete mixing.
13. Compare the color developed in specimen wells to that of the 20 ng/ml Standard Reference well.

INTERPRETATION OF RESULTS

1. Positive: Wells showing blue color stronger than the 20 ng/ml Reference Standard indicate the presence of AFP, or positive results.
2. Negative: Wells showing no color or faint blue color weaker than the 20 ng/ml Reference Standard indicate non-detectable amount or less than 20 ng/ml of AFP in the specimen.

A slight bluish tinge, much lighter than the 20 ng/ml Standard Reference well, may result from insufficient washing and should be considered negative.

Depending on the concentration of AFP in the specimen, the color may develop instantaneously.

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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TECHNICAL CONSULTATION

Call or Write: **BioCheck, Inc.**
323 Vintage Park Dr.
Foster City, CA 94404
Tel: (650) 573-1968 Fax: (650) 573-1969

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