

# AFP EIA kit

Enzyme-linked immunosorbent assay for the detection of Alpha-Fetoprotein (AFP) in Human Serum

Catalog #: CT3002

## INTENDED USE

AFP Enzyme Immunoassay test kit is intended for the qualitative and semi-quantitative determination of AFP concentration in human serum.

## SUMMARY AND PRINCIPLE OF THE TEST

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.

The AFP ELISA Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-AFP antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-AFP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the AFP antibody coated microtiter wells. If human AFP is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and AFP antibody labeled with horseradish peroxidase (conjugate) are added. The conjugate will bind immunologically to the AFP on the well, resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N H<sub>2</sub>SO<sub>4</sub>, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of AFP is directly proportional to the color intensity of the test sample.

## REAGENTS

### Materials provided with the kits:

1. 8X12 well microtiter strip: 1 plate, coated with anti-AFP.
2. Negative Control: One vial of AFP Negative Control.
3. Cut Off Reference: 25ng/ml.
4. Enzyme Conjugate: HRP-conjugated-anti-AFP for 96 tests.
5. Wash Buffer Concentrate (20 x): 40 ml for 96 tests, containing PBS, Tween. The buffer should be diluted 20 times with distilled water before use.
6. Substrate Solution A: 6 ml urea peroxide.
7. Substrate Solution B: 6 ml TMB.
8. Stop Solution: 6 ml 1N Sulfuric Acid

### Materials required but not provided:

1. Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader with 450nm wavelength
8. Timer

## PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Do not use kit beyond expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipet reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

## SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

## STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

## ASSAY PROCEDURE

1. Allow all components to reach room temperature before use.
2. Dispense one drop (50 ul) of Cut Off Reference, Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control., and 50ul of serum or plasma samples into respective wells
3. Add 50ul Enzyme Conjugate to each well, but **DO NOT ADD ENZYME CONJUGATE TO BLANK WELL.**
4. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
5. Wash each well 4 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
6. Add one drop (50 ul) of Substrate Solution A (HRP substrate) to each well, then add one drop (50 ul) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 15 min.
7. Add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

## INTERPRETATION OF RESULTS

**EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):**

Positive: sample OD ≥ Cut Off Reference OD  
Negative: sample OD < Cut Off Reference OD

### For semi-quantitative test:

Serial dilution of the specimen and the approximate titer will correspond to the highest serum dilution that still presents a positive result.

AFP (ng/ml) = titer \* 25ng/ml

## LIMITATIONS OF THE ASSAY

1. AFP EIA is limited to the detection of AFP in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of the entire content of wells before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made only on the basis of a single test. A complete evaluation by physician is needed for a final diagnosis.

## RELATED READING MATERIALS

1. Abelev G I. Alpha-fetoprotein as a marker of embryo-specific differentiation in normal and human tissues. Transplant Rev 1974;20:3-37.
2. Sell L S. Cancer markers of the 1990s. Clin Lab Med 1990;10:1-37.
1. 3. Hirai H, Nishi S, Watabe H et al. Some chemical, experimental and clinical investigations on alpha fetoprotein. In: Hirai H, Miyaji T, eds. Alpha-fetoprotein and hepatoma. Gann Monogr 1973;14:19-34.

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FOR CLINIAL USE

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