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CALBIOTECH
A life science company

Alpha-Fetoprotein (AFP) ELISA

Catalog No. AF237T (96 tests)

INTENDED USE

The Calbiotech AFP ELISA Kit is intended for the quantitative measurement of AFP in human serum.

SUMMARY AND EXPLANATION

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

This AFP ELISA kit is a solid phase sandwich assay method, based on a streptavidin-biotin principle. The standards, samples and the biotinylated Anti-AFP antibody reagent are added into designated wells, coated with Streptavidin. Endogenous AFP in the patient's serum binds to the antigenic site of the biotinylated Anti-AFP antibody. Simultaneously, the biotinylated antibody is immobilized onto the wells through the high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of the Peroxidase (HRP) conjugated Anti-AFP antibody reagent, a sandwich complex is formed, the analyte of interest being in between the two highly specific antibodies, labeled with Biotin and HRP. Unbound protein excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of AFP in the samples. A standard curve is prepared relating color intensity to the concentration of the AFP.

MATERIALS PROVIDED		96 Tests
1.	Microwell coated with Streptavidin	12x8x1
2.	AFP Standard: 6 vials (ready to use)	0.5ml
3.	Anti-AFP Enzyme Conjugate: 1 bottle (ready to use)	12ml
4.	Anti-AFP-Biotin Reagent: 1 bottle (ready to use)	12ml
5.	TMB Substrate: 1 bottle (ready to use)	12ml
6.	Stop Solution: 1 bottle (ready to use)	12ml
7.	20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25° C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 25 µl of AFP standards, control and patient's sera.
3. Add 100 µl of Anti-AFP-Biotin Reagent to all wells and mix for 20-30 seconds.
4. Cover the plate and incubate for 30 minutes at room temperature (20-25° C).
5. Remove liquid from all wells. Wash wells three times 300 µl with 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of the Anti-AFP- Enzyme conjugate to all wells. Cover and incubate for 30 minutes.
7. Remove liquid from all wells. Wash wells three times 300 µl with 1X wash buffer. Blot on absorbent paper towels.
8. Add 100 µl of TMB substrate to all wells.
9. Incubate for 15 minutes at room temperature.
10. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
11. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check AFP standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached**.**
2. To construct the standard curve, plot the absorbance for the AFP standards (vertical axis) versus the AFP standard concentrations in ng/ml (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Data

	OD 450 nm	Conc. ng/mL
Std 1	0.020	0
Std 2	0.072	5
Std 3	0.281	25
Std 4	0.462	50
Std 5	1.878	250
Std 6	2.447	500

LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.