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Product Information

Alpha-Fetoprotein (AFP) ELISA kit

Catalog Number: EA100884
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

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

Background

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 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 is a direct solid phase sandwich ELISA method. The samples and diluted anti-AFP-HRP conjugate are added to the wells coated with MAb to beta subunit. AFP in the patient's serum binds to anti- AFP MAb on the well and the anti-AFP second antibody then binds to AFP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of AFP in the samples. A standard curve is prepared relating color intensity to the concentration of the AFP.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell coated with AFP MAb	12x8x1
2. AFP Standard: 6 vials (ready to use)	0.5 ml
3. AFP Enzyme Conjugate: 1 bottle (ready to use)	12 ml
4. Incubation Buffer: 1 bottle	12 ml

5. TMB Substrate: 1 bottle (ready to use)	12 ml
6. Stop Solution: 1 bottle (ready to use)	12 ml
7. 20X Wash concentrate: 1 bottle	25 ml

Materials and Equipment Required but 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

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. 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.

Reagent Preparation

1. **Wash Concentrate:** Prepare 1X Wash buffer by adding the Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- 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.
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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 Incubation buffer to all wells and mix for 20-30 seconds.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26°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 Enzyme conjugate to all wells. Cover and incubate for 30 minutes.
7. Remove liquid from all wells, and repeat the washing process as in step 5.
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 Curve

	AFP (ng/ml)	OD450
Std 1	0	0.041
Std 2	5	0.147
Std 3	25	0.490
Std 4	50	0.735
Std 5	250	1.696
Std 6	500	2.285

Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for AFP may be used as initial guideline ranges only:

- AFP Normal Range = Less Than 20 ng/ml

Sensitivity: 0.348 ng/ml

References

1. Bates SE. Clinical applications of serum tumor markers. Ann Intern Med 1991;115:623-8.
2. Wu JC, Lee SD, Hsaio KJ, et al. Mass screening of primary hepatocellular carcinoma by alpha-fetoprotein in a rural area of Taiwan-a dried blood spot method. Liver 1988;8:100-4.
3. Lee H-S, Chung YH, Kim CY. Specificities of serum alpha-fetoprotein in HBsAg+ and HBsAg- patients in the diagnosis of hepatocellular carcinoma. Hepatology 1991;14:68-72.
4. Di Bisceglie AM, Rustgi VK, Hoofnagle JH, Dusheiko GM, Lotze MT. Hepatocellular carcinoma. Ann Intern Med 1988;108:390-401.
5. Sato Y, Nakata K, Kato Y, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha- fetoprotein. N Engl J Med 1993;328:1802-6.
6. Deutch HF. Chemistry and biology of alpha- fetoprotein. Adv Cancer Res 1991;56:253-312.