

Product Information

Alpha-Fetoprotein (AFP) ELISA

Catalog Number **SE120142**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Alpha fetoprotein (AFP) is a glycoprotein with a molecular mass of ~70,000 Da. 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 Alpha-Fetoprotein (AFP) ELISA Kit is intended for the quantitative measurement of AFP in human serum. 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 the beta subunit. AFP in the 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 the wash buffer. Upon addition of the substrate, the intensity of color is proportional to the concentration of AFP in the samples. A standard curve is prepared correlating color intensity to the concentration of the AFP.

Components

Materials Provided	96 Tests
Microwell coated with AFP MAb	12 x 8 x 1
AFP Standard: 6 vials (ready to use)	0.5 mL
AFP Enzyme Conjugate: 1 bottle (ready to use)	12 mL
Incubation Buffer: 1 bottle	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents 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 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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.
5. Do not use grossly lipemic specimens.

Reagent 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 (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that serum samples be run in duplicate.

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

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Prior to assay, allow reagents to stand at room temperature (18–26 °C). 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 sera.
3. Add 100 µL of the Incubation Buffer to all the 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 the wells. Wash the wells 3 times with 300 µL of 1x Wash Buffer. Blot on absorbent paper towels.
6. Add 100 µL of the Enzyme Conjugate to all the wells. Cover and incubate for 30 minutes.
7. Remove liquid from all the wells, and repeat the washing process as in step 5.

8. Add 100 µL of TMB Substrate to all the wells.
9. Incubate for 15 minutes at room temperature.
10. Add 50 µL of Stop Solution to all the wells. Shake the plate gently to mix the solution.
11. Read absorbance on an ELISA Reader at 450 nm within 15 minutes of adding the Stop Solution.

Results**Calculations**

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 the value is checked on every kit.
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 fit curve through the points.

Example of a Standard Data

	OD 450 nm	Concentration ng/mL
Std 1	0.041	0
Std 2	0.147	5
Std 3	0.490	25
Std 4	0.735	50
Std 5	1.696	250
Std 6	2.285	500

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

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: <20 ng/mL

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

References

1. Bates, S.E., Clinical applications of serum tumor markers. *Ann. Intern. Med.*, 1991, 115, 623-8.
2. Wu, J.C. 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. et al., Specificities of serum *alpha*-fetoprotein in HBsAg+ and HBsAg- patients in the diagnosis of hepatocellular carcinoma. *Hepatology*, 1991, 14, 68-72.
4. Di Bisceglie, A.M. et al., Hepatocellular carcinoma. *Ann. Intern. Med.*, 1988, 108, 390-401.
5. Sato, 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, H.F., Chemistry and biology of alpha-fetoprotein. *Adv. Cancer Res.*, 1991, 56, 253-312.

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