



## AFP ELISA

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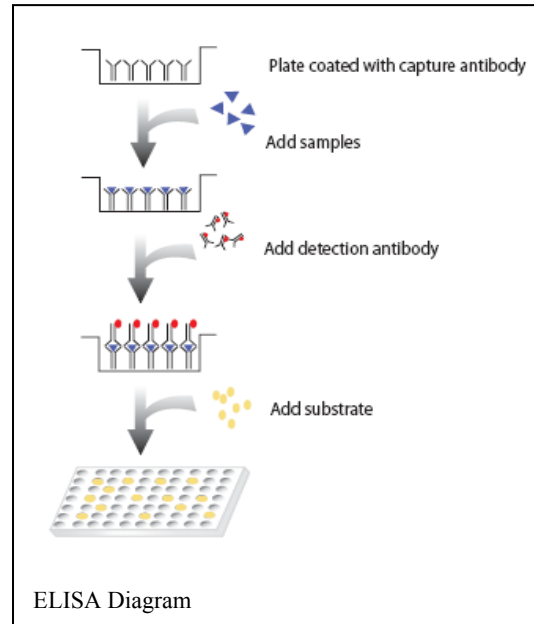
### Introduction

AFP is a glycoprotein with a molecular weight of between 65,000 and 70,000 daltons including 4% of carbohydrate (1). During fetal development, AFP produced in the vitelline (yolk) sac, fetal liver and in the fetal gastrointestinal tract, and maintains high levels in the serum, playing an important role in pregnancy monitoring. Throughout the remainder of life, AFP drops to very low levels, only small amounts of AFP can be detected in the blood of healthy adults. Rise of the AFP titer in serum may be pathognomonic for different clinical pictures, AFP is elevated in the malignant diseases of hepatocellular, testicular nonseminomatous origin, and occasionally of other entodermal origin (2,3,4). AFP may be slightly elevated or persisted in the patients with large hepatic metastases or viral hepatitis (5). AFP measurement is widely accepted as tumor marker and for monitoring the therapeutic effectiveness of hepatocellular cancer and nonseminomatous testicular cancer.

The Signosis AFP Immunoassay provides a rapid, sensitive, and reliable assay for the quantitative measurement of AFP level. The antibodies developed for the test will determine a minimal concentration of 2.0 ng/ml.

### Principle of the assay

The AFP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a goat anti-AFP antibody directed against intact AFP for solid phase immobilization (on the microtiter wells). A monoclonal anti-AFP antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with these antibodies, resulting in AFP being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of AFP is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



### Materials provided with the kits:

- Antibody-coated microtiter plate with 96 wells
- Zero Buffer, 13 ml.
- Reference standard set, contains 0, 5, 20, 50, 150, and 300 ng/ml (WHO, 72/225) AFP, lyophilized.
- Enzyme Conjugate Reagent, 18 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

### Materials required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

## Specimen Collection and Handling

Serum should be separated from the red blood cells as soon as possible. Specimens should be stored for up to 48 hours or -20°C for up to 6 months prior to assay.

## Preparation for Assay

- All reagents should be taken to room temperature (18-25 °C) before use.
- 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.

## Assay Procedure

Samples of patient serum, plasma need to be diluted before use for best bet results

1. Add 20 µl of standard, specimens, and controls into appropriate wells.
2. Add 100 µl of Zero Buffer into each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at room temperature (18-25°C) for 30 minutes.
5. Remove the incubation mixture by flicking plate content into a waste container.
6. Rinse and flick the microtiter wells 5 times with distilled or deionized water. Put the wells sharply onto paper towel to remove all residual water droplets.
7. Add 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
8. Incubated at room temperature for 30 minutes.
9. Remove the incubation mixture by flicking plate contents into a waster container.
10. Rinse and flick the microtiter wells 5 times with distilled water
11. Tap the plate sharply onto paper towel to remove residual water droplets.
12. Dispense 100 µl TMB Reagent into each well, and mix gently for 10 seconds.
13. Incubate at room temperature in the dark for 20 minutes.
14. Add 100 µl Stop Solution to each well to stop the reaction.
15. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
16. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

## References

1. Engall, E., Methods in Enzymology, Volume 70, Van Vunakis, H. and Langone, J. J. (eds.), Academic Press, New York, 419-492(1980).
- Uotila, M., Ruoslahti, E. and Engvall, E., J. Immunol. Methods, 42, 11-15 (1981).
2. Abelev G I. Alpha-fetoprotein as a marker of embryo-specific differentiation in normal and human tissues. Transplant Rev 1974;20:3- 37.
3. Hirai H. Alpha fetoprotein. In: Chu T M, ed. Biochemical markers for cancer. New York: Marcel Dekker, 1982:23-59.
4. Chan D W, Miao Y C. Affinity chromatographic separatoin of alpha- fetoprotein variants: Development of a mini-column procedure and application to cancer patients. Clin Chem 1986;32:2143-2146.
5. 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.

## Example of standard curve

AFP (ng/ml)	Absorbance (450 nm)
0	0.012
5	0.127
20	0.455
50	0.952
150	2.150
300	2.932

