POINTE SCIENTIFIC, INC.
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Alpha-Fetoprotein (AFP) Enzyme Immunoassay Test Kit

Intended Use
For the quantitative determination of Alpha-Fetoprotein (AFP) in serum. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Principle of the Test
The AFP ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a rabbit anti-AFP antibody directed against intact AFP for solid phase immunobilization (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 first with the immobilized rabbit antibody for 30 minutes. The wells are washed to remove unbound antigen. The monoclonal-HRP conjugate is then reacted with the immobilized antigen for 30 minutes at room temperature resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is 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.

Reagents
Materials provided with the kit:
- Rabbit anti-AFP coated microtiter plate with 96 wells.
- Zero Buffer, 13 mL
- Reference Standard Set, contains 0, 5, 20, 50, 150 and 300ng/mL, 0.5 ml each, ready to use.
- Enzyme Conjugate Reagent, 18 mL
- TMB Reagent (One-Step), 11 mL.
- Stop Solution (1N HCl), 11 mL.

Storage of test Kit and Instrumentation
Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

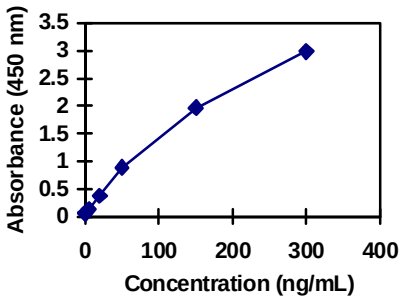
Reagent Preparation
1. All reagents should be brought to room temperature (18-25°C) before use.

Assay Procedure
1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Zero Buffer into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
13. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 100 µl TMB Reagent into each well. Gentle mix for 10 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read the optical density at 450 nm with a microtiter plate reader ***within 15 minutes***.

Calculation of Results
1. Calculate the average absorbance values (A_{450}) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of AFP in ng/mL from the standard curve.

Example of Standard Curve
Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against AFP concentrations (ng/ml) shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

AFP (ng/ml)	Absorbance (450 nm)
0	0.053
5	0.126
20	0.378
50	0.891
150	1.968
300	2.986



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