Assay Solution
A Solution Provider

Alpha-Fetoprotein (AFP) Enzyme Immunoassay Kit

Cat# ART- IA1001

Name	**AFP ELISA**
Full name	**Human AFP (Alpha Fetoprotein) ELISA Test Kit**
Category Name	Cancer ELISA kits
Catalog number	ART-IA1001
Test	96
Method	ELISA method: Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-300 ng/mL
Sample	20ul serum, plasma with EDTA/heparin, cell culture supernates
Specificity	98.5%
Sensitivity	2.0 ng/mL
Total Time	~80min
Shelf Life	12 Months from the manufacturing date

Intended Use

AFP Enzyme Immunoassay test kit is intended for the quantitative determination of AFP concentration 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.

Entrez Gene IDs	174 (Human); 11576 (Mouse); 24177 (Rat)
Alternate Names	AFP; Alpha-1-fetoprotein; alpha-fetoglobulin; alpha-Fetoprotein; DSCAM2; FETA; HP; HPAFP
NCBI Accession Number	NP_001125.1
Swiss Prot Number	P02771
Subcellular location	Secreted

Assay Principle

The AFP Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-AFP antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-AFP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the AFP antibody coated microtiter wells and incubated with the Zero Buffer. If human AFP is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and AFP antibody labeled with horseradish peroxidase (conjugate) are added. The conjugate will bind immunologically to the AFP on the well, resulting in the AFP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After an incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the

color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of AFP is directly proportional to the color intensity of the test sample.

Sample Collection and Storage

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Material and Components

Materials provided with the test kits

1. Antibody-coated microtiter plate with 96 wells.
2. Zero buffer, 12 ml.
3. Reference standard set, contains 0, 5, 20, 50, 150, and 300 ng/ml AFP, in liquid form (ready to use) or lyophilized form.
4. Enzyme Conjugate Reagent, 18 ml.
5. TMB Substrate, 12 ml.
6. Stop Solution, 12 ml.
7. Wash Buffer Concentrate (50X), 15ml
8. Controls set (optional)

Materials required but not provided

1. Precision pipettes: 5~40 µl, 50~200 µl and 1.0 ml.
2. Disposable pipette tips.

3. Distilled water.

4. Vortex mixer or equivalent.

5. Plate washer, absorbent paper or paper towel.

6. Graph paper.

7. Microtiter plate reader.

Reagent Preparation

1. All reagent should be brought to room temperature (18-22^{o}C) before use.

2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

3. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 20µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of zero buffer into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-22°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container, or using a plate washer.
7. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 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 washing buffer (1X).
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100µl TMB substrate into each well. Gentle mix for 5 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100µl of stop solution to each well.
17. Gently mix for 30 seconds to make sure that the blue color changes to yellow color completely.

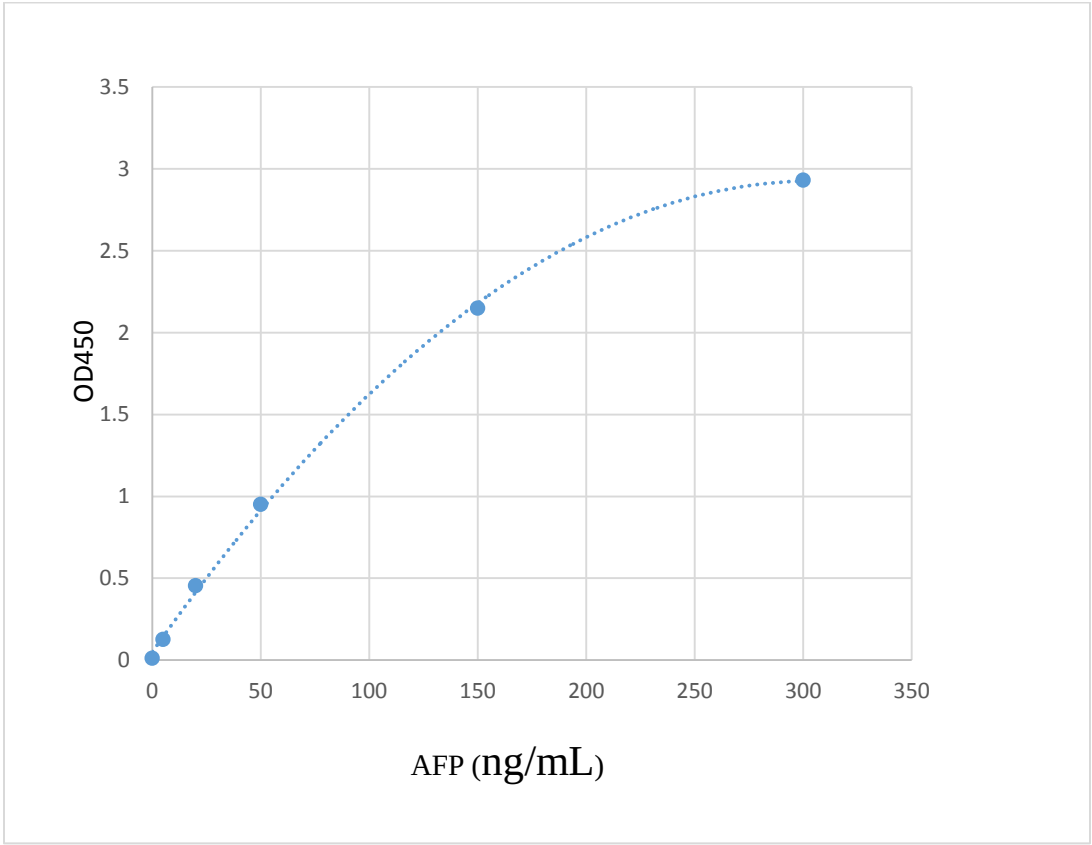
18. Read optical density at 450nm with a microtiter reader within 15 minutes.

Important Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Results

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper or using softmax, Grappad, Microsoft Excel, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of AFP in ng/ml from the standard curve.

Example of standard curve
Results of typical standard run with optical density reading at 450nm shown in the Y-axis against AFP concentrations 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.



Sample values and sensitivity

In high-risk patients, AFP values between 100 and 350 ng/ml suggest a diagnosis of hepatocellular carcinoma, and levels over 350 ng/ml usually indicate the disease. Approximately 97% of the healthy subjects have AFP levels less than 8.5 ng/ml. It is recommended that each laboratory establish its own normal range. The minimum detectable concentration of AFP by this assay is estimated to be 2.0 ng/ml.

Performance

I. Accuracy:

Comparison between our Kits and Commercial Available Kits provides the following data:

N = 79
Correlation Coefficient = 0.985
Slope = 1.038 Intercept = 0.729
Mean (Our) = 55.12
Mean (Abbott) = 52.24

II. Precision:

1) Intra-assay

Concentrations	N	Mean	S.D.	%CV
Group1	24	31.04	1.45	4.66
Group2	24	126.8	5.2	4.1
Group3	24	270.8	12.09	4.78

2) Inter-assay

Concentrations	N	Mean	S.D.	%CV
Group1	24	30.58	1.88	6.1
Group2	24	125.1	7.08	5.7
Group3	24	268.3	14.06	5.2

III. Linearity

Two patient sera were serially diluted with 0 ng/mL standard in a linearity study. The average recovery was 102.2 %.

IV. Recovery

Various patient serum samples of known AFP levels were mixed and assayed in duplicate. The average recovery was 101.1 %.

V. Cross-reactivity

The following human materials were tested for crossreactivity of the assay.

Limitation of the assay

There are some limitations of the assay:

1. As with all diagnostic grade tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

Storage

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

Troubleshooting Information

High Background

Probable Cause:	Solution/ Action
High incubation temperature:	Incubate at room temperature (25 ^{o}C) throughout the procedure
Insufficient washing of the plate:	Fill the wells with wash buffer and aspirate completely for the next wash Increase the number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, if available and check that all the channels are operating properly
Concentrated streptavidin-HRP	Streptavidin-HRP was not diluted properly Dilute the streptavidin-HRP as mentioned in the manual
Light exposure during substrate incubation	The TMB substrate is light sensitive and turns to blue color in the presence of light. The incubation must be carried out in dark.
Stop solution not added	Color will continue to develop if stop solution is not added
Diluents came with the kit were not used	Standards/ sample, detection antibody and streptavidin-HRP must be diluted in the respective buffers came with the kit. Do not use buffers from other kits
Contaminated solutions	Prepare fresh working solutions

Poor Standard Curve

Probable Cause:	Solution/ Action
Improper standard reconstitution:	Spin the vial briefly before opening Reconstitute the standard as mentioned in the manual. After reconstitution, leave it atleast for 10 minutes at room temperature Do not store and reuse diluted standards
Curve fitting problem:	Log transform the values on both axes Use 4-PL/ 5-PL curve fitting programs
Incubation temperature/ time	Use the recommended standard incubation conditions
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.

No Signal

Probable Cause:	Solution/ Action
Omission of reagent(s):	Read the manual entirely. Check that all the reagents are added in the correct order as stated in the manual
Incorrect detection antibody was used:	Use the detection antibody came with the kit
Chromogen solutions were mixed improperly	Use the recommended procedure to prepare the TMB substrate
HRP inhibitor in sample/ buffers	Check that the samples/ buffers do not have sodium azide as it will inhibit peroxidase reaction.
Vigorous washing	If the washing is done manually, pipette the wash buffer gently.
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations
Improper plate reader settings	Check the wavelength and read the plate again

Erratic duplicate OD values

Probable Cause:	Solution/ Action
Insufficient washing of the plate	Fill the wells with wash buffer and aspirate completely for the next wash Increase number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, is available and check that all the channels are functioning properly
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.
Improper mixing of samples/ buffers	Mix the samples well before pipetting Thoroughly mix the working solutions of detection antibody/ streptavidin-HRP
Contamination from other wells	Do not reuse the adhesive covers from previous assay setups Change pipette tips during reagent addition. If same pipette tip is being used to dispense reagents, care should be taken, not to touch the solution in the well
Precipitates in the samples/ buffer	If precipitates are visible in wash buffer concentrate, keep it at 37 ^{o}C for 10-15 minutes until no precipitates are visible Centrifuge the samples to remove particulate matter
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations